THE JOURNAL OF PHYSIOLOGY

EDITED FOR

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VOL. 81

1934

CAMBRIDGE UNIVERSITY PRESS
LONDON: FETTER LANE, E.C. 4

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PRINTED IN GREAT BRITAIN

8113

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THE ELECTRICAL RESPONSES OF LIGHT- AND DARK-ADAPTED FROGS' EYES TO RHYTHMIC AND CONTINUOUS STIMULI.

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(*Received December* 29, 1933.)

PART I.

Working with the well-known phenomena of flicker and fusion, Schaternikow [1902] noted that the fusion frequency of a flickering light was lowered by dark-adaptation; and this finding has been amply confirmed by Lythgoe and Tansley [1929]. Some experiments by Piper [1911] indicated that the fusion frequency of ripples on the retinal action potential, caused by intermittent light, was lower in animals possessing chiefly rods (cat) than in those having chiefly cones (pigeon); but to our knowledge nobody has investigated the retinal response to intermittent light in light- and dark-adaptation with an eye which has both types of receptors present in considerable numbers. This consideration led us to undertake work along these lines with the frog's eye. The retina of this animal is well supplied with both rods and cones, and it was found to survive fairly well in excised eyes.

Well-marked differences in the behaviour of light- and dark-adapted eyes reacting to continuous and intermittent stimuli were noted, and these proved to be analysable in terms of the components of the retinal action potential [Granit, 1933]. Part I gives a brief description of the results obtained with continuous light. Part II contains an experimental analysis of the mechanism of "flicker" (intermittent ripples), made possible by the finding of a method for producing intermittent ripples, as it were, *in statu nascen.*

Technique.

The records have been obtained with the permanent magnet string galvanometer and directly coupled amplifier previously used by one of us [Granit, 1933]. The stimulus was a concentrated filament lamp with a condenser lens, both inside a metal case with a circular opening of

10 mm. diameter at a distance of 35 cm. from the eye. The intensity of the light was about 1800 metre candles, as measured directly with a Lummer-Brodhun contrast photometer at the distance used. In a few cases neutral tint filters were used (Tscherning glasses); but all records published or described below have been obtained under conditions of maximal stimulation.

Eyes of large Hungarian frogs (*Rana esculenta*) or of *R. temporaria*, which is common here, were used indiscriminately as they were found to behave apparently identically. The frogs to be dark-adapted were placed in the photographic dark room from one to several hours; then the eyes were quickly excised below a dark red lamp and put on a specially constructed ebonite holder inside a shielded and earthed light-proof box. The eyes were supported from behind by a pad of cotton wool, soaked in Ringer solution. The wick of a non-polarizable electrode was taken to this pad. The ebonite holder contained a 4 mm. wide artificial pupil through the side of which the thin cotton wick of the corneal electrode was led. This artificial pupil was actually too large and served chiefly as a fixing device for the eye. As will be shown below, the differences between light- and dark-adapted eyes were too obvious to be explained by the diminished intensity resulting from the contraction of the pupil in the light-adapted state, and, besides, were of such a nature, in passing from dark- to light-adaptation, as would otherwise have indicated an increased intensity of stimulation in the light-adapted state. The quantitative work in Part II was carried out with a light-adapted eye and thus with a pupil of constant size.

The stimulus entered the box through a narrow blackened tube, serving as a light trap in the feebly illuminated room. In the light beam were placed: a circular rotating metal shutter, weighted by a metal rod, an episcotister with two 90° open sectors, and the edge of a prism reflecting part of the beam through other prisms to the camera. A slit in the circular shutter could be divided into parts so as to produce one or several flashes at various intervals. For approximately constant standard exposures of 2 sec. the shutter was operated by hand to the sound of a metronome. The episcotister was geared to a large wheel, usually rotated by hand, but operated by a load when comparable exposures were wanted in a series of experiments.

With eyes in the dark-adapted state 2 sec. exposures were taken at intervals of 3 min. Light-adaptation was obtained by allowing the stimulating light to shine all the time; then before the experimental observation the shutter was closed for a few seconds, during which the

off-effect (see below) disappeared. The response to the same stimulus was thus obtained against a "background" of darkness just as with the dark-adapted eye. The interval of darkness was too short for the slowly reacting pupil to change in size.

Bulbs, thoroughly cleaned from adjoining tissue, were found to give reasonably constant responses for 30–40 min. and slowly diminishing ones for a considerably longer time. In the course of this work some 700 responses have been analysed, all of which have been from frogs giving the normal type of response at the beginning of the experiment.

Results.

Normal responses to continuous illumination.

As is well known from the comparative studies of v. Brücke and Garten [1907], Piper [1911], and others, the retinal action potentials of all vertebrate eyes are similar in outline. The first reaction to illumination

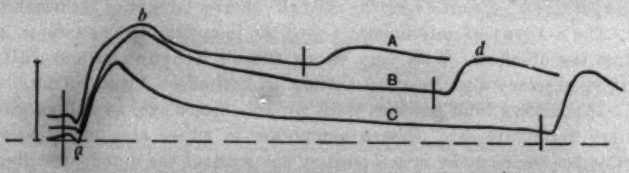

Fig. 1. The short vertical lines show the beginning and end of the stimuli: 1 sec. in *A*; 1·5 sec. in *B*; 2 sec. in *C*. Explanation in text. In this and subsequent figures the vertical line to the left shows calibration to 0·673 mv.

is the negative dip, *a*, then follows a positive rise, the *b*-wave, which at high intensities fairly rapidly drops, to be succeeded by a slow secondary rise, the *c*-wave. Finally, on cessation of stimulation there is a positive off-effect or *d*-wave. These features, with the exception of a definite *c*-wave, may be recognized in the responses shown in Fig. 1, which are taken at fairly fast speeds of the film and with relatively short exposures.

A is obtained after 4 hours' dark-adaptation. The potential remains fairly high after the *b*-wave proper and would have risen still higher into a typical *c*-wave, had the film been allowed to run further. In the frog's eye the *c*-waves are so slow that we have observed them only by inspection. The fact to be stressed is that this secondary rise disappears with light-adaptation [cf. v. Brücke and Garten, 1907, and several others later]. In the last response, *C*, the eye is completely light-adapted, and

the curve slopes downwards towards a constant low level of potential, typical for the light-adapted eye. The *b*-wave has diminished. Another still more striking difference between light- and dark-adapted eyes is the increase in the rate of rise and in the size of the off-effect, which increase runs parallel with a shortening of its latent period. Thus *B* is taken 3 min. after *A* (the eye being in the dark meanwhile), and though the exposure is only 0·5 sec. longer than in *A*, this has definitely influenced the off-effect. In the thoroughly light-adapted eye the off-effect changes less rapidly with the length of the exposure. Even with short exposures in the light-adapted eye there are fairly brisk off-effects, while they can hardly be seen at all with the dark-adapted retina.

The initial *a*-wave may or may not be bigger in the light-adapted eye. Its size will depend upon how the negative *a* and the positive *b* balance against one another. In certain responses (see Fig. 2, this time after massage), which to all appearance are normal, the *a*-wave may be preceded by a small positive hump, indicating a shorter latent period of the positive *b* compared with the normally shorter latency of the negative *a*. The *a*-wave certainly increases with the intensity of the stimulus, as does the off-effect. With very strong stimuli Einthoven and Jolly [1908] observed *a*-waves of 0·3–0·6 mv. and off-effects up to 1·0 mv.

It is known from previous work with the frog's eye, as well as with other vertebrate eyes, that a diminution in either area or intensity simplifies the response into a positive one without the details described above [cf. a review by Kohlrausch, 1931]. As to the differences with light- and dark-adapted eyes, our work chiefly confirms earlier observations summarized by Kohlrausch.

In the retinal action potentials of frog's eyes certain features which indicate a more complicated picture have been described by Chaffee, Bovie and Hampson [1923]. They found in some eyes definite small humps on the *b*-wave. In rare cases we also have seen such humps. It should be observed, however, that these authors used excised eyes with opened bulbs and considerable amplification. Under such circumstances the electrodes are near enough to the optic nerve to enable the amplifier to pick up the discharge in the nerve. Before we conclude that such phenomena are retinal more definite evidence appears to be needed.

The components of the retinal action potential.

The retinal action potential of the vertebrate eye (cat) has recently been split into components [Granit, 1933]. A positive component, P I, could be easily removed with narcotization of the animal and was found

to be chiefly responsible for the *c*-wave. The initial *b*-wave appeared as part of another positive component, P II, which then continued hidden under the *c*-wave. The *a*-wave was found to be the initial phase of a negative component, P III, which in normal responses continued below the positive components, and, with its relatively quick return towards the base line, elicited the off-effect by algebraical summation with the slower after-discharge of the positive components. The three components were found as three stages in progressive narcotization, and P II, besides, could be removed selectively through asphyxiation of the animal.

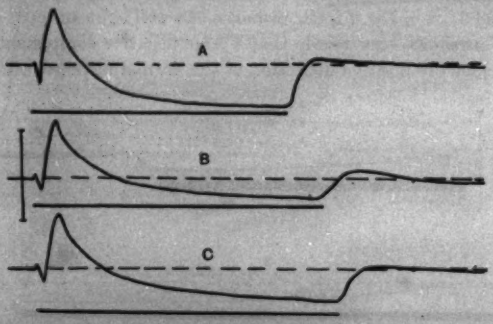

Fig. 2. Unbroken horizontal lines show duration of the stimuli: 1·8 sec. in *A*; 2 sec. in *B*; 2·1 sec. in *C*. Broken horizontal lines show respective base lines of the galvanometer. Explanation in text.

Apart from the time relations the only difference between the frog's and the cat's retinal response is seen in those features, *a*-wave and off-effect, which were shown to be dependent chiefly upon the negative P III. Both *a*-wave and off-effect are bigger in the frog's eye. We shall now see whether this is sufficient reason for assuming that the analysis established with the cat's eye is invalid for the frog.

The slow secondary rise, as we saw, can easily be removed by light-adaptation. Similarly in the cat's eye this rise disappears with light-adaptation [Granit, unpublished observations, confirming Kahn and Löwenstein, 1924, with the rabbit's rod-eye]. It is, however, not so easy to remove selectively other phases of the response of the frog's retina as it is with the warm-blooded mammalian eye. Instead of the quick, reversible changes produced by ether or asphyxia in the cat's eye, there appear in the cold-blooded retina very slow irreversible alterations

in the general type of response, indicating that two components are affected simultaneously.

A definite step forward was taken when, utilizing an old observation by Waller [1909], we began to massage the eyes. After massage Waller obtained a pure negative deflection, with a fairly quick initial drop possessing the latent period of the *a*-wave, and a very quick rise towards the base line on cessation of stimulation. We have not succeeded in obtaining such negative waves entirely free from positive humps, but relatively pure negative responses may at times be had in a few minutes by this method. The characteristics of such potentials are illustrated by Figs. 2 and 3. *A* in Fig. 2 is the response of a well light-adapted eye after massage. It shows very clearly that a large negative component may be hidden under the positive deflection of the normal response and that its

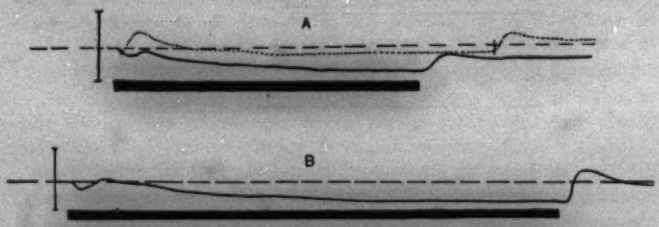

Fig. 3. Stimuli for curves drawn in full shown by thick black lines: 2·6 sec. in *A* (the dotted line is the response to a stimulus of 3·2 sec. ending at the short vertical line); 4·3 sec. in *B*. Explanation in text.

rise at the end towards the base line is very fast. It also shows that the large rise at "off" may take place entirely on the negative side of the base line. The eye was then given 5 min. in the dark, after which response *B* was obtained. The positive initial rise is unaltered, but the negative deflection is now considerably smaller and rises more slowly towards the base line. The eye was light-adapted once more for 2 min. and the response, *C*, again gives a larger negative deflection with a brisk rise towards the base line. Throughout this experiment the *b*-wave is practically constant and the changes with light- and dark-adaptation are reflected only by the behaviour of the negative wave.

It can be shown in another way that the rise at "off" is relatively independent of the *b*-wave. In Fig. 3*A* the upper dotted response is obtained immediately after massage of a light-adapted eye. Later the response has assumed the shape traced below in full. Despite considerable

diminution in the size of the *b*-wave the amount of rise at cessation of stimulation remains equal in the two responses. The total reaction is merely somewhat slower. That the *b*-wave and the off-effect may alter independently has also been shown by Nikiforowsky [1912] and Tirala [1917]. We have also seen above that with light-adaptation the *b*-wave diminishes and the off-effect increases (cf. Fig. 1). The last

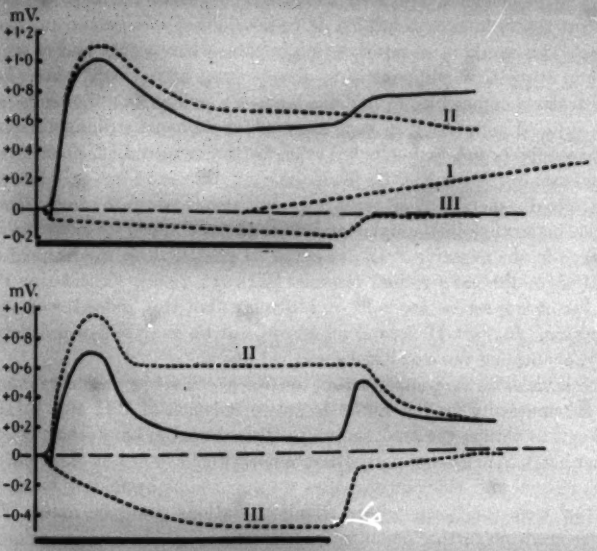

Fig. 4. Analysis of the components of the retinal action potential (drawn in full) in dark-adaptation (upper set of curves) and in light-adaptation (lower set of curves). Components drawn in broken lines. Thick black lines indicate a stimulus of 2 sec. duration. Explanation in text.

response of Fig. 3 shows a considerable negative deflection concurrent with a small *b*-wave. In such responses the negative may or may not rise above the base line at "off"; however, we have reason to believe that a pure negative deflection would show a quick initial swing towards the base line at cessation of stimulation, but that it would remain below it and gradually approach it asymptotically in what we have termed a "negative remnant." Signs of such a negative remnant can be seen in all the responses of Figs. 2 and 3, best marked in the upper curve drawn

in full in Fig. 3, and also, at times, in normal responses. The long negative remnants may be, partly at least, pathological. That the negative deflection itself is a normal component of the retinal response is shown by Kohlrausch's observations with coloured lights and the pigeon's eye [1918]. Partly negative responses may be obtained by altering the wave-length of the stimulating light.

The phenomena described above indicate that the negative component reacts in such a fashion as to necessitate a large and rapid off-effect. This would be of positive sign in cases where at the end of stimulation a positive component is disappearing at a slower rate. It is particularly convincing to find the negative component to change with the state of adaptation so as to account for the most striking difference between light- and dark-adapted eyes, *i.e.* the increased off-effect and its rapid rate of rise (cf. Fig. 1). Thus the chief difference, not only between the retinal responses of cats and frogs, but also between the frog's retinal action potentials in the light- and dark-adapted state, follows from the change in the negative P III, as could be predicted on the basis of the analysis of the cat's retinal response [Granit, 1933]. Considering that all vertebrate retinæ are built on a similar plan this, indeed, was to be expected. In Part II normal off-effects will be analysed in a different way, confirming the conclusions reached above.

Our views on the components of the frog's retinal response are summed up schematically in Fig. 4. As complete isolation of P II and P III is difficult to obtain, the analysis can hardly pretend to be precise. On the other hand, in constructing the diagrams of Fig. 4 to fit our observations with normal and deficient responses we were struck by finding how very narrow were the limits within which alterations could be made. The figure needs no further comment.

Discussion.

Before proceeding to an experimental analysis of the changes with intermittent light, to which Part II will be devoted, there is one fact of general significance in the theory of light- and dark-adaptation which these experiments have emphasized. This is that light- and dark-adaptation involve a great deal more than a mere change in the amount of visual purple available in the rods. Through all these experiments we have been using the same stimulus, both for producing light-adaptation and for stimulation, and this stimulus has been far above the thresholds for both rods and cones. Why then do not the cone effects appear superimposed upon the rod effects in dark-adaptation? The most

obvious change with light-adaptation is an increase in the negative P III, which increase, if present, would be bound to appear in a dark-adapted eye upon the smaller P III of that state of adaptation. In terms of current theories on the physiology of the retina, working exclusively with over-simplified concepts as to the functional properties of rods and cones, our results are inexplicable. Obviously light-adaptation involves a fundamental change in the reaction of the eye as a whole. During continued illumination the eye is being "set" in a different fashion; the change might well be elicited by way of the paths activated through the initial reaction to light in the cones. A cone eye seems to be "set" in this fashion from the beginning, to judge from some results obtained by Meservey and Chaffee [1927]. These authors studied the responses of cold-blooded eyes possessing chiefly cones (turtle, horned toad, chameleon) and compared them with the responses of frogs' eyes. The off-effect was found better marked in the cone eye, which also appeared to lack a *c*-wave [but see Kohlrausch, 1918, for warm-blooded cone eyes]. The *b*-wave appeared to rise more rapidly in the cone eye.

PART II.

The responses to intermittent illumination in light- and dark-adapted eyes.

Fig. 5 *A* shows the retinal action potential of a thoroughly dark-adapted eye (4 hours in the dark room). It is noticeable that despite the considerable potential developed and the rapidity of its development there are nevertheless at the most only traces of intermittent waves during the course of interrupted stimulation. The eye was then illuminated for 5 min. and after the off-effect had disappeared the record *B* was obtained. The briskness of the waves that now appear is striking and again indicates a fundamental change in the reaction of the retina. The string in both cases is at the same tension, as shown by the calibration to the left of *A* (0·67 mv.). The fusion frequency in *B* is at about 14 flashes per second. With less thoroughly dark-adapted eyes than the one responsible for curve *A* values between 6 and 8 flashes per second may be obtained.

The response *C* begins with the off-effect after some minutes of light-adaptation. Then intermittent stimulation follows before the off-effect has had time to drop. Several features in this record should be noticed: (i) that the first effect of the first flash upon the off-effect is to cause a

large negative dip followed by a spontaneous rise before the next flash appears; (ii) that as the rate of intermittent stimulation increases the whole curve swings downwards, and again swings upwards at the end of the curve when the stimuli are spaced farther apart; (iii) that the peaks of the upstrokes are very sharp; and (iv) that there are small ripples superimposed upon the rounded tops of two long flashes in the middle of the curve. The fusion frequency is at about 18 flashes per second. In this experiment the eye was next dark-adapted again for 15 min., and the

Fig. 5. Responses to intermittent light. Time marking given by tuning fork (1/100th sec.). Stimuli, directly photographed, shown below tuning fork record. Explanation in text.

fusion frequency fell to 7; after 8 min. of renewed light-adaptation it once more rose to 18.

Already in 1908 Einthoven and Jolly had noticed that particularly large *a*-waves were obtained when a second stimulus followed soon after interruption of the first. Piper [1911] seems to have overlooked this observation, though he was aware of the fact that the first effect of a new stimulus, when small "gaps" in a continued illumination were produced, always was the typical negative dip (frog's eye) initiating reactions to light.

Below we have undertaken a systematic study of this and other phenomena relating to the behaviour of the off-effect. The method has

been to superimpose flashes upon the off-effect of a continuous stimulus of approximately constant duration. From the point of view of "flicker" the theoretical basis for such a procedure should be evident. An illuminated eye is, as it were, prepared to discharge its off-effect as soon as the stimulus is interrupted. If the flashes are sufficiently far apart the off-effect will be discharged. These are the conditions with which we are dealing in interrupting the illumination so as to cause intermittent waves.

As will be seen below the phenomena are complicated, and we shall therefore begin by stating briefly what the experiments show. The analysis will be carried out for the light-adapted eye before comparisons are made with the dark-adapted retina. It will be demonstrated that the negative component, P III, plays a decisive rôle in the production of intermittent ripples, that at rates of intermittent stimulation near the fusion frequency the off-effect is all-important, but that with slower rates of stimulation and very brisk flicker a *b*-wave plays an increasingly bigger part. Finally, spontaneous continuation of rhythmic activity after cessation of stimulation will be shown to occur under certain conditions.

Results with flashes superimposed upon the off-effect.

(*a*) *Flashes of constant duration at various intervals after cessation of the initial stimulus.*

Fig. 6 shows the effect of a flash of about 40σ superimposed upon the off-effect of a light-adapted retina at various intervals after cessation of stimulation. The vertical line through all the curves marks the end of a 2 sec. stimulus, the small vertical lines on the individual curves show the onset of the flash. The off-effect has gradually diminished during the experiment, but this does not interfere with the general trend of the result. This is easily confirmed by trying the various intervals in haphazard order, though for a rapid survey involving several observations it is convenient to run them in series. In every case the first effect of the flash is to cause a negative dip which is small at short and at long intervals, and largest when at the top of the off-effect. Farther out this

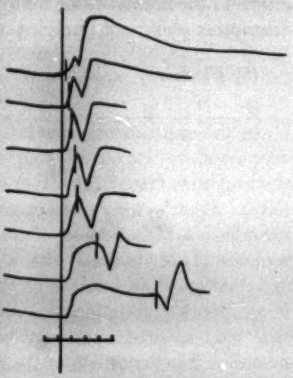

Fig. 6. Time marking shown = 1/10th sec. Explanation in text.

flash also causes a brisk positive peak which clearly is above the level of the off-effect. Though the film is run at slow speed one further effect is obvious enough: this is the lengthening of the latent period of the negative dip at short intervals.

If the amount of negative potential caused by the flash at different intervals after the 2 sec. stimulus is given as a percentage of the average uninterrupted maxima of the off-effects of one whole series, values are obtained which, plotted against time as abscissæ, fall along curves of the type shown by the lines drawn in full in Figs. 7 and 8. As zero time is taken the point at which the uninterrupted off-effect would reach its maximum. The average curve for development and decay of the off-effect itself is given in dotted lines in the same figures. The curves in Fig. 7 are obtained with a very active eye giving large negative dips; Fig. 8 represents a less active eye, but the negative dip is traced farther out to a point where it has reached the normal size of the *a*-wave in this particular eye during light-adaptation. It is thus shown to change gradually into a normal *a*-wave, a conclusion substantiated by other observations below. To the left of the starting point of these curves no negative dips are obtained. These early flashes cause merely an initial retardation of the rate of rise of the off-effect. Just after illumination there is, as it were, an "absolute refractory period" for *a*-waves, then follow a "relatively refractory period," a "supernormal phase," and a return to the normal value for *a*-waves. These terms are used in a purely descriptive sense, not implying any analogy with peripheral nerve.

(*b*) *Flashes of variable duration at various intervals after cessation of stimulation.*

In the experiments illustrated in Fig. 9 the off-effect proved to be very constant. Its normal course is illustrated in broken lines in most of the responses traced. The lines drawn in full show the responses themselves. Again every curve begins with the last phase of a 2 sec. constant stimulus. A higher speed of the film is used than in Fig. 6. The first response (1) is obtained with a 40σ flash following the interruption of illumination at an interval of 40σ. This, if repeated, would amount to intermittent stimulation at a rate of 12 $\frac{1}{2}$-flashes per second. Values in well light-adapted eyes vary between 12 and 20 for the fusion frequencies. The first effect of the flash is the typical negative dip; but the important point demonstrated by this experiment refers to the rise following the negative. The off-effect continues after the interrupting negative dip and reaches its "intended" value. Thus the effect of the

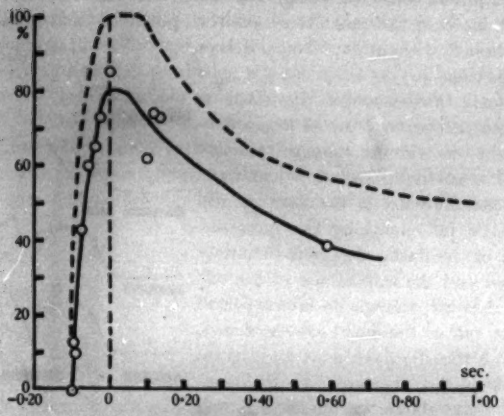

Fig. 7. Average latent period of off-effect (dotted curve) is 40σ. Explanation in text.

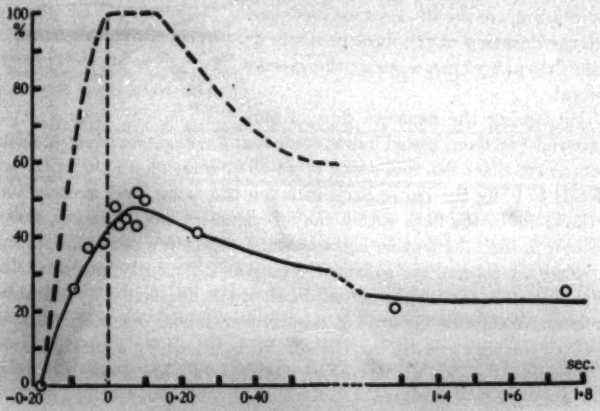

Fig. 8. Average latent period of off-effect (dotted curve) is 50σ. Explanation in text.

flash is a purely negative swing. The additional stimulus of the flash does not increase the amount of positive potential that would have been obtained without it; it only delays its rate of rise.

As soon, however, as the flash is lengthened, in curve 2 to 110σ, it definitely adds to the amount of positive potential that the off-effect would have given alone. The size of the negative dip is but little influenced by the increased duration of the flash. But it is definitely broadened and the rate of rise of the off-effect is delayed, though its latent period (from the end of the flash) has shortened. In curve 3 the 40σ flash is at an interval of 230σ from the cessation of the illumination. The rise above the off-effect is still more marked. In curve 4 the flash is at the same interval, but greatly lengthened. The negative is uninfluenced but the positive swing above the off-effect has increased with the duration of the flash precisely as in the first pair of responses at the shorter interval.

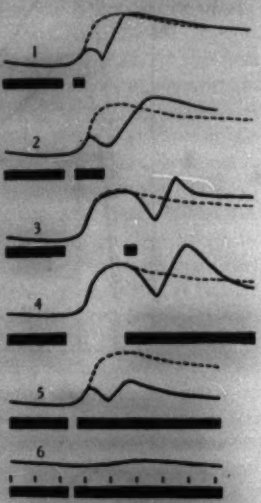

Fig. 9. Time marking shown = 1/10th sec. Stimuli indicated by thick black lines. Explanation in text.

Considering the negative dips of this figure and Fig. 6 one would like to state that the negative effect of flashes upon an off-effect has something of an all-or-none character in the way it tends to bring the string back to much the same level irrespectively of the locus of the flash within the off-effect. Seeing, however, that in addition to the off-effect an extra amount of positive potential appears, provided the flashes are sufficiently long or sufficiently late in the off-effect, one realizes that the actual depth of the negative dip need not be a correct measure of the level of negativity reached. Farther out on the off-effect the negative dip may be counterbalanced by a positive swing of short latent period and involving more than a mere resumption of the off-effect.

Curve 5 shows that with a sufficiently long flash it is even possible to obtain a small positive rise at as short an interval as 38σ, and this rise can hardly be an off-effect. In the last response with a long flash at the very short interval of 22σ both negative and positive are considerably

flattened out and the stimulus acts as if directly continued. These, however, are not the conditions in which we are interested.

We want to know to what extent a positive swing which is not an off-effect can play a rôle in producing ripples in the retinal action potential in flicker. To this end we plot as ordinates the amount of rise **above** the off-effect, elicited by short flashes, against time as abscissæ (Fig. 10). In order to exclude slight variations during the course of the experiment the difference between the total positive swing and the height of the off-effect is given in percentage of the *b*-wave of the 2 sec. stimulus (the

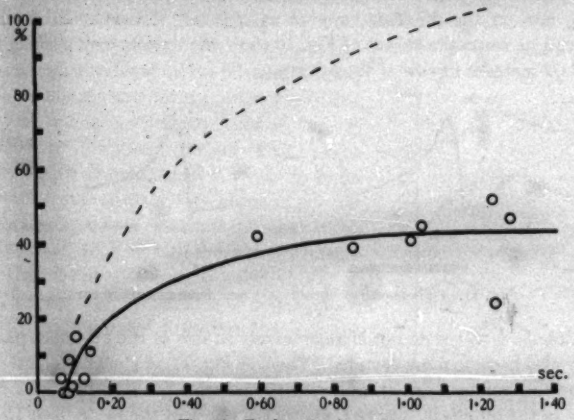

Fig. 10. Explanation in text.

positive peaks are below shown to be *b*-waves). Zero time in this figure is the point at which the off-effect is maximal (about 230σ from cessation of stimulus), and which is shown by the broken vertical line in Figs. 7 and 8. The curve drawn in full is based on observations from the experiment which provided the negatives plotted in Fig. 8. The flashes are 35σ long. The broken line shows the average curve from five experiments with flashes of longer duration (240σ). It rises more steeply than the line drawn in full, in accordance with the fact that longer flashes cause larger positive swings. Together both curves show that with flashes at the rates necessary for small flickering ripples in the retinal action potential, that is, just below the fusion frequency, a positive swing which is **not** an off-effect does not enter into the question. The true positive is effective only

when rates of intermittent stimulation are used which allow the off-effect to reach its plateau height. Now, the very shortest time measured in our experiments from the cessation of the constant stimulus to the top of the off-effect is 120σ. Usually it is much longer, say, about 200σ. Within this time brisk flicker may occur before any other positivity than the rising off-effect has had a chance to develop. This conclusion will be substantiated by other evidence below (section (*g*)).

Just as the negative dips caused by short flashes on the off-effect gradually diminish in size along the off-effect and become ordinary *a*-waves, so the positive peaks gradually increase in size and, after some time, late on the off-effect, appear as ordinary *b*-waves. In a purely descriptive sense the curves of Fig. 10 show the "refractory period" and gradual increase in size of the *b*-wave.

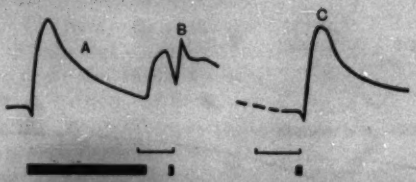

Fig. 11. Time markings shown = 0·5 sec. Explanation in text.

But the *b*-waves do not change merely in size as the off-effect passes. They also become more rounded. Thus, in Fig. 11, *A* shows the potential of the continuous 2 sec. stimulus; *B* the positive peak elicited by a flash early in the off-effect. Curve *C* shows the effect of the same flash 10 sec. after cessation of a stimulus similar to *A*. The off-effect has then disappeared. The motor running the camera has picked up speed during this long interval as shown by the broadening of the flash and by the lines marking an interval of 0·5 sec. But the positive peak, *C*, is broadened far more than can be accounted for by the increased speed of the film. This effect upon the shape of the positive peaks is more marked with shorter flashes such as are used in intermittent stimulation.

(*c*) *The effect of two successive flashes upon the off-effect.*

In curve 1, Fig. 12, there is again the typical effect of a single flash (50σ); in curve 2 the first flash is followed by another one (35σ) at an interval of 35σ. The negative dip is slightly increased in size, but still more obvious is the absence of ripples corresponding with the flashes.

The rate of rise of the positive swing following the flashes is slowed. In curve 4 the pair of flashes is shifted farther inwards on the off-effect. The broadened negative and the delayed rate of rise of the positive swing is more marked than in curve 2. In curve 5, however, where though the interval between the flashes is again 35σ they occur earlier still, there are definite ripples corresponding with them. In Fig. 14 this effect is shown by original photographs. The first flash of curve 5, Fig. 12, is at an interval of only 25σ from the end of the constant stimulus. In curve 4 the corresponding interval is 40σ. In curve 6 this interval is 30σ but the flashes are only 20σ apart.

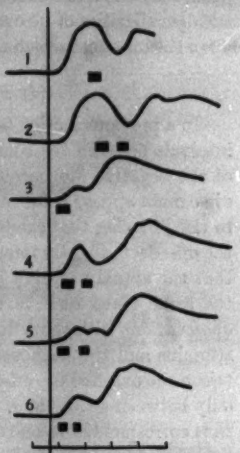

Fig. 12. Time marking shown = 1/10th sec. Explanation in text.

It is clear then that double flashes at short intervals apart well out on the off-effect merely tend to bring about a return of the conditions set by a constant stimulus, that is, an eye prepared to discharge its off-effect afresh together with an additional *b*-wave. In order to obtain ripples during this phase of the off-effect a slower rate of intermittent stimulation must be used, such as with the first two flashes upon the off-effect shown by curve *C*, Fig. 5. Then, exceedingly brisk flicker may appear, as both the increased negative dips and the positive peaks above the level of the off-effect, described in the previous sections, have time to develop.

In order to obtain flicker at fast rates the off-effect must be interrupted by a small negative dip from which it can recover without a very much delayed rate of rise. Then a second early flash can interrupt it a second time (curve 5, Fig. 12). This condition is automatically brought about as the rate of intermittent stimulation increases. The optimal conditions for ripples at fast rates thus tend to "reset" themselves by bringing the eye back to the state at which the off-effect is held spring-like ready to go. And now we understand why with flicker on an off-effect the total level of potential can be made to swing up and down with the rate of stimulation as in curve *C*, Fig. 5. At slow rates off-effects and *b*-waves together push the string upwards in the picture, whereas at fast rates the negative dips quickly cut down the off-effect and the *b*-waves and sum to bring about a low potential near the base line, on which ripples

appear as interrupted off-effects. How under such conditions two negative flashes sum to keep the string down may be seen in curve 4, Fig. 12.

The small ripples occurring after the double stimuli in curves 2, 4, and 6 in Fig. 12 cannot be explained as artefacts. Below it will be shown that, although it is possible to derive the details of retinal flicker at various rates of stimulation from the behaviour of the *a*-wave, the off-effect and the *b*-wave, yet, as soon as a definite rhythmic response is produced, this tends to persist for some time as a definite rhythmic state with peculiarities of its own. At times this state is produced after flashes upon the off-effect which themselves have not been able to set up ripples.

(*d*) *Factors determining fusion.*

In a previous section (*a*) it has already been pointed out that, at short intervals between the constant stimulus and the flash, the latent period of the negative dip becomes lengthened. This means that the negative dip cannot appear before a certain small amount of off-effect has had time to rise. Flashes that are too early merely retard the initial rate of rise of the off-effect; slightly later flashes have a relatively long latent period, so that the actual negative dips at short intervals tend to occur in much the same place relative to the off-effect. Thus in a series of twelve observations with short flashes at intervals between the end of the 2 sec. stimulus and the flash varying between 25 and 125σ, the intervals between the onset of the off-effect and the onset of the negative dips varied only between 40 and 90σ. A range of variation of 100 in the former case thus corresponded to one of 50 only in the latter set of values. Too rapid flashes, relative to the latent period, therefore keep the string down without causing ripples.

In the last instance then, the latent period of the off-effect and its rate of rise determines the fusion frequency. This is clearly seen when for some reason or other the eye becomes less active, as happens in every experiment with excised eyes after some time. Then the latency of the off-effect increases, its rate of rise decreases, and the fusion frequency diminishes. But it is particularly instructive and convincing to demonstrate the effect of the lengthened latency and the decreased rate of rise of the off-effect by dark-adapting the eye or by decreasing the strength of the stimulus with filters.

(*e*) *Comparison between dark- and light-adapted eyes.*

In Fig. 13, *A* and *B* are the responses of a dark-adapted eye and show the slow, late off-effect. A calibration to 0·67 mv. is shown to the

left in the figure. The eye is then light-adapted and the sensitivity of the instrument is increased so as to make the two responses, *C* and *D*, in this state of adaptation, practically equal to *A* and *B*. The flashes in *A* and *C* occur after an interval of 120σ, in *B* and *D* after an interval of 270 and 290σ respectively. In the dark-adapted eye the first effect of the flash, inasmuch as it has any effect at all, is to cause a negative dip, but both this and the rise following are less marked than in the light-adapted eye. Curve *A* in particular, being the first response of an eye left for a long time in the dark-room, shows that the negative dip requires an off-effect as a background against which to appear. The results are so obvious that further comment seems unnecessary.

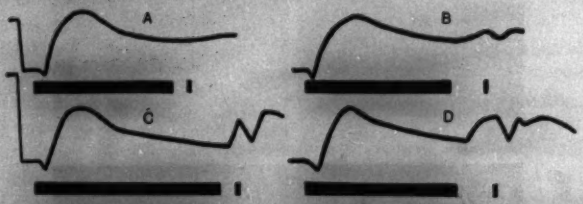

Fig. 13. Stimuli shown by thick lines. *A*, initial stimulus lasts 1·3 sec. and is followed at an interval of 120σ by a flash of 35σ; *B*, corresponding values for stimulus and interval are 1·3 sec. and 270σ; *C*, corresponding values 1·6 sec. and 120σ; *D*, corresponding values 1·3 sec. and 290σ.

It is easy to see why flicker in the dark-adapted eye requires slow rates of intermittent stimulation. Brisk flicker requires a brisk off-effect. We are thus brought back, in our analysis of intermittent stimulation, to the same difference between light- and dark-adapted eyes which we learned to recognize when using steady illumination. Just because the mechanism of flicker in light- and dark-adapted eyes is the same, the intermittent waves and the fusion frequency decrease in the latter state of adaptation.

(*f*) *Spontaneous rhythms.*

As indicated by curves 2, 4, and 6, Fig. 12, as well as by the response *C* of Fig. 5, small rhythmic waves in no direct relation to the stimuli themselves may appear after rhythmic stimulation. In the beginning we believed them to be artefacts, though, on the other hand, we were aware of the fact that Einthoven and Jolly [1908] described spontaneous rhythms in the retinal action currents from frogs, and that Adrian and Matthews [1928] recorded in the optic nerve of the conger eel rhythmic

grouping of impulses with continuous stimulation and found the phenomenon to be similar to the waxing and waning of impulses obtained with intermittent stimulation [cf. also Adrian, 1932]. Application of strychnine to the eye seemed to make the retina especially prone to set up spontaneous rhythmic discharges through the nerve, and this tendency was particularly well marked when the whole retina was illuminated. In the beginning we ended most experiments with application of strychnine under various conditions of area, intensity, and dose of

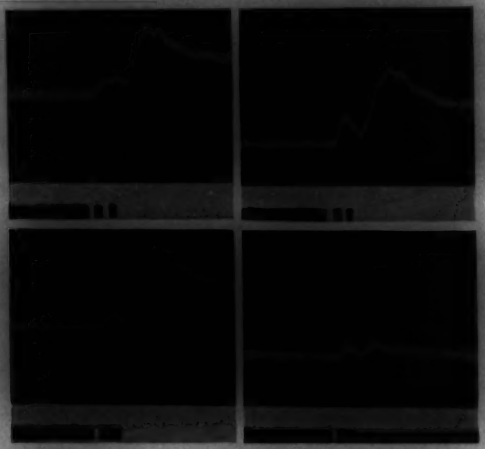

Fig. 14. Spontaneous rhythms upon off-effects interrupted by flashes. As in Fig. 5. Explanation in text.

the poison, but did not succeed in obtaining spontaneous rhythms. Probably the time of the year is of some importance, as Adrian and Matthews obtained this effect most easily in the summer.

However, spontaneous rhythms have often appeared under conditions illustrated by the responses of Fig. 14. Starting a flicker with two flashes at short intervals from the cessation of the 2 sec. stimulus leads to continued ripples on the off-effect at much the same rate, between 13 to 14 flashes per second in response *A*. The second response, *B*, shows that the flashes can appear too late on the off-effect to cause anything but a negative dip, at this interval apart, and yet be followed by spontaneous

ripples, gradually damped out as the off-effect passes. The conditions illustrated in *C* are less favourable to the setting-up of spontaneous rhythms. And with experimental conditions of the type illustrated in response *D* they have not appeared. The off-effect must be given a chance to develop. At times there is some indication of a rhythm even on an uninterrupted off-effect, though we find that in eyes where spontaneous rhythms tend to occur, a short interval and one or two flashes distinctly favour the appearance of them. It is as if a taut string were sharply plucked.

(*g*) *Experiences with deficient responses.*

This point need not detain us long, as in every respect the observations with deficient responses have confirmed conclusions reached in previous sections. We shall therefore only summarize the results:

(i) If the positive components are largely removed in such a fashion as to leave a mainly negative response with a brisk off-effect towards the base line, a flash upon this off-effect gives the typical negative dip, this time practically down to the original level of negativity reached by the steady stimulus. The whole effect may take place on the negative side of the base line and shows that the negative dip merely represents a resumption of the previous value of P III. Hence it increases when the off-effect increases.

(ii) P II is necessary for obtaining the positive peaks above the level of the off-effect, just as it is necessary for obtaining the *b*-waves. These sharp positive peaks above the level of the off-effect diminish *pari passu* with the loss of the positive component initiated by the *b*-wave.

(iii) Provided that a brisk off-effect is left in a largely negative response, intermittent ripples are obtained even though the initial *b*-wave has become very small and rounded. The fusion frequency in such cases is lowered, which may, however, be merely because of the fact that in such cases the negative is less brisk than in an eye giving a response normal in every respect. At slower rates the sharp peaks of the *b*-waves are lacking and do not appear until the rate is slow enough to show them as rounded second tops on the quicker initial upstrokes caused by the interrupted off-effects. Such records emphasize in a most convincing way the double nature of the upstrokes in retinal flicker, set forth above.

(*h*) *Comparison with an eye dominated by rods.*

Piper [1911] held that in eyes with a large negative *a*-wave and a correspondingly large off-effect (frog) retinal flicker was chiefly determined by *a*-waves and off-effects and to a lesser extent by *b*-waves, as we

have proved above to be the case, whereas in eyes with small *a*- and *d*-waves (cat) the ripples consisted only of repeated *b*-waves. Creed and Granit [1933], working with the dark-adapted cat's eye, were prepared to admit that the *a*- and *d*-waves played a rôle in flicker, but did not find Piper's evidence conclusive. They took the view that the response to intermittent light could not be reconstructed from data provided by measurements of the latent periods of the various waves obtained with single isolated flashes and brief interruptions of a steady light (Piper's method). This criticism was based on the facts that an isolated *b*-wave tends to take its own course independently of whether it is elicited by one or several flashes [Piper, 1911; Creed and Granit, 1933], and that in the cat's eye the latency of the intermittent ripples is longer than that of the initial *b*-wave. Moreover, Creed and Granit found it unlikely that various vertebrate eyes should behave in a fundamentally different fashion with respect to intermittent stimulation. Finally, they produced some evidence to the effect that in the cat's eye also interaction between *a*-waves and *b*-waves must be assumed in order to account for certain features of the response to intermittent stimulation. A definite negative dip, as the first effect of intermittent flashes, could not be seen, but the latency of the upstroke was lengthened in the intermittent positive peaks as compared with the initial value, and this was interpreted as due to a negative swing temporarily balancing the upstroke.

In the frog's eye the latent periods change in a complicated fashion with the rate of stimulation, thereby emphasizing the difficulty in basing conclusions on identification of the latent periods under various conditions. Above, most of our measurements of latencies have been disregarded, as our conclusions have been definite enough without them. But in view of the work of Creed and Granit one set of measurements might be mentioned. If with ripples near the fusion frequency the interval from the beginning of the flash to the upstroke following the negative dip is measured, a value is obtained which corresponds to the one measured by Creed and Granit in the cat's eye. This they called the latent period, obviously enough, as the upstroke was the first measurable effect of the flash. The flash length at the fusion frequency was found to be about 44 p.c. of this "latent period" independently of how the experimental conditions were varied as to area and intensity. In the light-adapted frog's eye we have obtained a similar constant by averaging thirty determinations of the flash length at the fusion frequency at various intensities together with the corresponding "latent periods." The value works out as 40 p.c. with a mean variation of 9·4 p.c. of 40. The result indicates a

similar mechanism for flicker and fusion in the eyes of cats and frogs. On the other hand, it is quite probable that the relative parts played by P II and P III in flicker are in favour of P II with the cat's eye. Its off-effect is small, and removal of P II leads to disappearance of the intermittent ripples [Creed and Granit]. Again, the fact that a few isolated flashes lead to a single *b*-wave without ripples is understood when one realizes that with stimuli of short duration the negative P III is small;

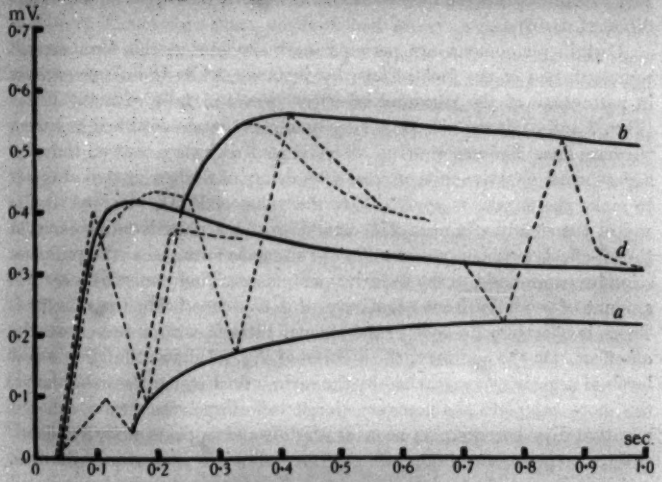

Fig. 15. Explanation in text.

it is perhaps too small to interact so as to produce ripples. Moreover, in the cat's eye the off-effect becomes better marked when the eye is left for some time in the light.

Discussion.

Certain facts concerning the origin and development of the rhythmic reaction to intermittent stimuli have emerged from this analysis with unmistakable directness. These are conveniently summarized by Fig. 15. The figure represents data obtained in a typical experiment with single flashes upon the off-effect and shows the levels of potential reached by the off-effect (*d*-wave), by the negative dips (*a*-wave), and by the positive peaks (*b*-waves). Curve *d* is the average off-effect. Curve *a* gives the level of the negative dips occurring along the off-effect, as indicated by the

broken lines showing the effects of actual flashes. Finally curve *b* represents the height of the positive peaks as again shown by the broken lines. The latter curve begins, as we have seen above, at the point where the off-effect is about to turn downwards. The evidence with both normal and deficient responses agrees in demonstrating that the *a*-, *b*- and *d*-curves express the behaviour of the waves in the retinal action potential, known for descriptive purposes as the *a*-, *b*- and *d*-waves. It is evident that Fig. 15 summarizes the results shown in Figs. 7, 8, and 10 in somewhat different plottings.

Certain phenomena are perhaps more obvious in this summarized representation of the facts. Thus, for instance, while the *a*-curve given in percentage of the maximal off-effect rises and falls with the latter (Figs. 7 and 8), it here changes very much less in absolute height above the base line. The experiments with deficient responses showed that the supernormal negative dips express a tendency of flashes on the off-effect to make the negatives approximate the value of P III, attained at the end of the steady stimulus. The smallness of the negative dips early in the off-effect means only that a flash of the same strength as the previous stimulus cannot add to the negative component. There must be a certain amount of recovery from negativity, this recovery being the off-effect. There is therefore no real "supernormal phase" with *a*-waves on the off-effect. On the contrary, the *a*-curve of Fig. 15 shows that the lowest levels of negativity are reached by the earliest flashes. On the other hand, the more complete the recovery (larger off-effect), the larger can the negative dips become. As soon as the *b*-wave appears above the off-effect, two processes of opposite sign are balancing against one another, and it is impossible to draw any conclusions as to the absolute size of either of them without knowing the latent periods and rates of rise of the individual components.

In contradistinction to the quick recovery of P III (off-effect) stands the slow recovery of P II, the earliest signs of this component appearing about 200σ after cessation of the 2 sec. stimulus (Figs. 10 and 15). The analysis of the components (Fig. 4) shows that at cessation of illumination P II must remain at plateau level for much the same period. Probably therefore the rise of the *b*-curve indicates the point where P II begins to drop from its plateau height and hence is prepared to rise again to a new stimulus of the same strength as the previous one.

Flicker at all but the fastest rates is obviously a process involving interaction between two opposite potentials in the retina. Though fast flicker can be obtained by interrupting momentarily the quick recovery

of P III alone, it is, on the other hand, probable that very fast negative components can be obtained only in eyes with some P II present. At least in the cat's dark-adapted eye no flicker appears with a pure negative response [Creed and Granit, 1933], and interaction between P II and P III must be assumed in order to account satisfactorily for a number of observations with intermittent light. As soon as flicker has been started it might well continue as a process *sui generis*, to judge from the fact that spontaneous rhythmic reactions sometimes appear upon intermittent stimulation. The curious sharp peaks of the *b*-waves on the off-effect and the delayed rate of rise of the off-effect, when interrupted by a long flash, may also be expressions of specific types of interaction between P II and P III.

Even more than the results with continuous light, the observations with intermittent illumination stress the fundamental change in the activity of the eye after light-adaptation. The dark-adapted eye is a sluggish organ, registering grossly the total amount of light. With the appearance of the large negative P III during light-adaptation, the retina has become a very rapid and delicate differential instrument recording the slightest change in the amount of luminous flux reaching the eye. This can be prettily demonstrated by passing filters quickly through the light beam. But the gradual alteration in the type of reaction, when a dark-adapted eye is left in the light, is most conveniently followed by observing the briskness of the intermittent ripples caused by intermittent stimulation and by noting the fusion frequency. In the introduction we mentioned that the equivalent sensory phenomenon, the fusion frequency as perceived by a human subject, changes with the state of adaptation just as the retinal fusion of flicker, studied above. This suffices to show that the effect, traced to the negative component of the retinal action potential, involves the physiology of our visual sensations, and thereby emphasizes the significance of possessing for a starting point in retinal and visual problems a formal analysis of the complex electrical reaction to light in the retina [Granit, 1933]. Obviously the current concepts as to the processes underlying light- and dark-adaptation must be revised in order to conform to the facts described above. This analysis will probably also shed light upon several other functions involving rapid and delicate retinal reactions, such as brightness discrimination and visual acuity. But the discussion of sensory phenomena will be postponed till a later occasion, when simultaneous records of impulses in the optic nerve should be available. The hypothesis that P III is associated with an inhibitory process [Granit, 1933] can then be directly tested.

SUMMARY.

The retinal action potentials of light- and dark-adapted frog's eyes have been recorded with a string galvanometer and a directly coupled amplifier. Part I describes the typical responses to continuous light and discusses their relation to the components of the retinal action potential [Granit, 1933]. Part II presents an analysis of the reaction to intermittent stimulation in terms of the various waves and components of the retinal action potential.

The chief changes during light-adaptation of an originally dark-adapted eye are: disappearance of the *c*-wave, diminution in the size of the *b*-wave, and a considerable increase in the size and rate of rise of the off-effect (*d*-wave) together with a shortening of its latent period.

Largely negative responses, produced by massage of the eye, show that the negative component, P III, on light adaptation increases in size, and on cessation of stimulation returns towards the base line with an increased rate of rise and thereby causes a large and rapid off-effect to take place wholly on the negative side of the base line. This effect can occur without concomitant changes in the *b*-wave and in P II.

Removal of P I (*c*-wave) and the change in P III suffice to explain the main differences between the responses in light- and dark-adapted eyes. Fig. 4 shows the components, P I, P II, and P III, of the retinal action potential of the frog's eye in light- and dark-adaptation. The increase in P III and its rapid return at "off" account for the increased off-effect of the light-adapted eye.

In the discussion to Part I it is shown that the increase in P III with light-adaptation makes untenable the widely held view that light-adaptation merely involves a shift from rod- to cone-vision following removal of visual purple. On the contrary the whole type of reaction of the eye has altered.

On the idea that an eye reacting to interrupted light is an eye prepared to discharge its off-effect at every interruption of the illumination, the responses to intermittent light have been analysed in the light-adapted eye by superimposing one or two flashes of short duration upon the off-effect of a constant 2 sec. stimulus. The first visible effect of such flashes is a negative dip, the size of which varies with the size of the off-effect as shown in Figs. 7 and 8. The negative dip is followed by a positive swing.

At short intervals between the end of the constant stimulus and the flash, the positive swing merely consists of an off-effect which has been momentarily interrupted by the initial negative dip. At longer intervals

the positive swing adds a sharp peak above the level of the off-effect. This peak increases with the interval and with the duration of the flash (see Fig. 8).

The negative dip is an *a*-wave, the positive peak above the level of the off-effect is a *b*-wave. As the off-effect passes they gradually become the typical *a*- and *b*-waves of the normal response. When P II is removed by massage the positive peaks and the *b*-waves disappear with P II. P III alone is needed for the *a*-waves and the negative dips, together with the off-effect, which the latter interrupt. The results with largely negative responses further show that the increased negative dips upon the off-effect express a tendency of a flash upon the off-effect to inhibit the latter and re-introduce the large negative P III attained at the end of the previous 2 sec. stimulus.

Intermittent ripples on the retinal action potential are produced by *a*-, *b*- and *d*-waves (off-effect). At fast rates of intermittent stimulation fast "flickering" ripples are produced by small *a*-waves interrupting the off-effect produced by P III returning towards the base line. At slow rates of intermittent stimulation the intermittent waves become very brisk owing to increased negative dips followed by positive peaks (*b*-waves) above the level of the off-effect.

In some eyes one or two suitably timed flashes upon the off-effect elicit a spontaneous rhythm which is gradually damped out.

In the dark-adapted eye the intermittent ripples are small and fuse at very slow rates of stimulation, less than 6 or 8 flashes per second. In the light-adapted eye the fusion frequency may reach 21 flashes per second and generally soon goes above 12.

Brisk intermittent ripples and a high fusion frequency presuppose a large P III and a correspondingly large and brisk off-effect. On the small, slow off-effect of the dark-adapted eye the positive peaks and negative dips, caused by single flashes, are much smaller than in the light-adapted eye.

The dark-adapted eye cannot follow quick changes of the illumination. It is a sluggish organ registering grossly the total amount of light. When the large and rapid negative component, P III, appears during light-adaptation, the retina becomes a very delicate and rapid differential instrument, as shown particularly by the brisk reaction to intermittent stimulation.

It is pointed out that this fundamental change in the behaviour of the eye is also demonstrated by the sensations. The fusion frequency, as perceived by a human subject, decreases with dark-adaptation just as does the fusion frequency of the retinal action potential.

We wish to tender our thanks to the Rockefeller Foundation for a grant (to R. G.) towards apparatus, to the neurosurgical clinic of the London Hospital for a grant (to L. A. R.), and to the Societas Scientiarum Fennica for defraying expenses in connection with this work.

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DIETETIC FACTORS INFLUENCING THE GLUCOSE TOLERANCE AND THE ACTIVITY OF INSULIN.

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(*Received* 23 *January*, 1934.)

It has long been recognized in the therapeutics of diabetes mellitus that the composition of the diet exerts a pronounced effect upon a diabetic's tolerance for carbohydrates, but it is only recently that attention has been drawn to the effect of different diets upon a healthy individual's tolerance for glucose. That starvation lowered the carbohydrate tolerance of a normal person was clearly recognized by Claude Bernard [1877], and it is noteworthy that he did not confuse such cases of glycosuria with cases of early diabetes mellitus or symptomless glycosuria (renal glycosuria), even though the only criterion of impaired or normal tolerance at his disposal was the presence or absence of glycosuria. Within the last ten years sporadic papers have appeared on the effect of diet upon this aspect of carbohydrate metabolism, and in 1927 Sweeney published results showing the effect of each class of foodstuff upon the glucose tolerance of healthy men. It is now established that the sugar tolerance is impaired by starvation or the taking of diets with a high content of fat, whilst it is improved by taking diets containing an excess of carbohydrate. This latter improvement would appear to be linked with a phenomenon observed by Hamman and Hirschman [1919], the so-called Staub-Traugott effect. These workers noticed that if the same dose of glucose were given at intervals of 90 min. the blood-sugar tolerance curve improved with each successive dose.

The effect of variations in diet upon insulin action has, until recently, been almost completely neglected. Abderhalden and Wertheimer [1924] and Bainbridge [1925] demonstrated that animals were more sensitive to insulin when receiving a carbohydrate diet than when they were eating a fat diet or were starving. Tiitso [1925–6] showed that, in starving animals, the blood sugar fell more slowly after insulin than in

those allowed a full diet. Recently Hynd and Rotter [1931] have demonstrated that there is a definite relation between the composition of the diet and the time after insulin injection that hypoglycæmic symptoms occur; animals on a high carbohydrate diet showed symptoms earlier than animals on a high fat diet.

It appeared to the writer that a correlation existed between the effect of diet upon the subsequent sugar tolerance and its effect upon the susceptibility of an animal to insulin, and it was shown, in healthy men [Himsworth, 1933], that those diets which caused an improvement in sugar tolerance also permitted a more rapid depression of the blood sugar by insulin, whilst those which produced a diminished sugar tolerance always retarded the rate at which the blood sugar fell after insulin injection. In the present paper it will be shown that identical results can be obtained on animals and that this inter-relationship, between sugar tolerance and insulin action, holds good in a wide variety of conditions.

Methods.

Male rabbits, approximately 2 kg. in weight, were used.

The *diets* given were of two kinds. The carbohydrate diet was composed of 250 g. of fresh cabbage and approximately 150 g. of a mixture of oats and bran in the proportion of 1 to 3. The fat diet was made up of 50 g. of fresh cabbage and 120 g. of soya-bean meal moistened with water. This amount of soya bean was about the maximum that a 2 kg. rabbit would eat in 24 hours. On analysis the soya-bean meal yielded 17 p.c. of ether soluble substances. A considerable proportion of this fraction was made up of lecithine, and it is of interest, in view of the recent work of Best and Hershey [1932], that its presence did not prevent the usual adverse effect of fat upon the sugar tolerance. Judged by human standards the fat diet contained a comparatively small proportion of fat, but judged by the standard of the diet habitually eaten by rabbits it will be seen that the fat bulks relatively largely.

All the animals receiving soya bean were of our own breeding, and were placed on this diet when they were 12 weeks old, but were not used for experimental purposes until they had reached the age of 6 months. The animals ate the soya bean with avidity and, although they appeared somewhat thinner than rabbits on a carbohydrate diet, their condition continued to be excellent. It should be noted that no experiment was performed on an animal until it had been taking the required diet for at least 2 months. All experiments were commenced at 10 a.m., 18 hours after withdrawal of food.

The *blood sugar* was estimated in 0·1 c.c. of whole blood by the Hagedorn-Jensen method.

Blood samples were taken from the marginal vein of the ear. As it was thought that vasomotor changes in the ear might affect the sugar content of the blood, by altering the rate of blood flow through the tissues, precautions were taken against this source of error. The animals remained at rest in a special box and were kept warm throughout the whole experiment, after previously having been accustomed to this lengthy procedure and also to the necessary handling. In the earlier experiments, 2 months before being used, the sensory nerves to both ears were cut and the superior cervical sympathetic ganglion on each side was removed. Later it was found that, provided the animals were well trained, and that they were kept comfortably warm, this precaution was unnecessary.

No attempt to obtain samples was made until the ear veins were fully distended with blood. A cut with a sharp Hagedorn needle was then made in the long axis of the vein, the specimen taken, and a small square of filter paper pressed lightly on to the cut for 30 sec., and then left in position. If the cut had been made cleanly, blood quickly ceased to escape and yet the flow continued undisturbed through the vein underneath. Removal of the filter paper with a squeezing sliding motion restarted the bleeding.

The times of sampling were decided before the experiment commenced. The operator was warned just previous to the scheduled time, and the exact second that the blood filled the pipette was noted by an assistant. In experiments after injection of insulin (insulin depression curves) samples were taken in the early part of the curve every 1½ min. and later every 3 min. In the different types of glucose tolerance curves the time and frequency of sampling varied, but in the same type of experiment the corresponding samples for each curve were taken at exactly the same minute.

The accuracy with which blood-sugar samples can be obtained by the above technique and the constancy of the results under the same conditions can be judged from Figs. 1 and 2.

Insulin injections were all given intravenously into the marginal vein of the ear not being used for blood sampling. For investigation of the effect of insulin upon the blood sugar the subcutaneous route is unsatisfactory owing to the slow and irregular absorption from the site of injection. A solution of *crystalline* insulin, 10 units per c.c., for a sterilized solution of which I am greatly indebted to Dr J. W. Trevan of the Wellcome Physiological Research Laboratories, was used in all experi-

ments, as commercial insulin had been found unsuitable, owing to its containing impurities which cause a transient initial hyperglycæmia on intravenous injection. In the insulin depression curves one-half unit of insulin was given by means of a syringe of fine bore accurately calibrated.

Glucose injections were given into the marginal vein of the ear from which blood was not being withdrawn. The glucose was made up as a 20 p.c. solution in normal saline and sterilized by boiling. At each injection 5 c.c. of this solution (1 g.) was given at a uniform rate which was

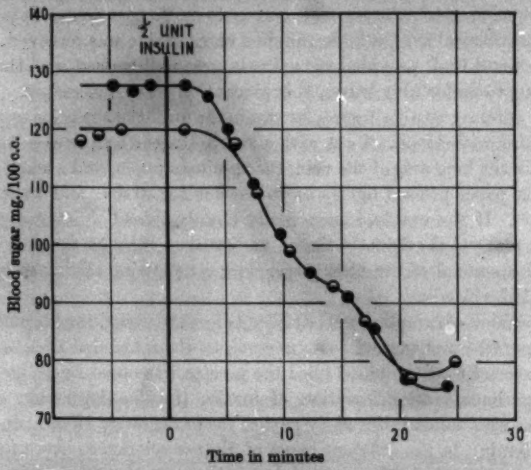

Fig. 1. Insulin depression curves after one-half unit of insulin obtained from the same animal, on two different occasions, whilst taking the carbohydrate diet.

timed so that the whole injection took one minute. As the animals were all of approximately the same weight no attempt was made to regulate dosage by surface area. In experiments in which consecutive doses of glucose were given (consecutive tolerance curves) all injections were carried out at the same time intervals. When insulin was injected in the course of a consecutive tolerance curve the dose chosen was 5 units, and it was given immediately before the last blood sample of one curve and 2½ min. before the next glucose injection.

Adventitious factors affecting blood-sugar curves after injection either of glucose or of insulin may either impair or improve the animal's capacity

to remove sugar from the blood. Excitement and hæmorrhage both interfere with the removal. The effect of excitement can be seen from Fig. 7. Any animal which, after two or three periods of training, did not

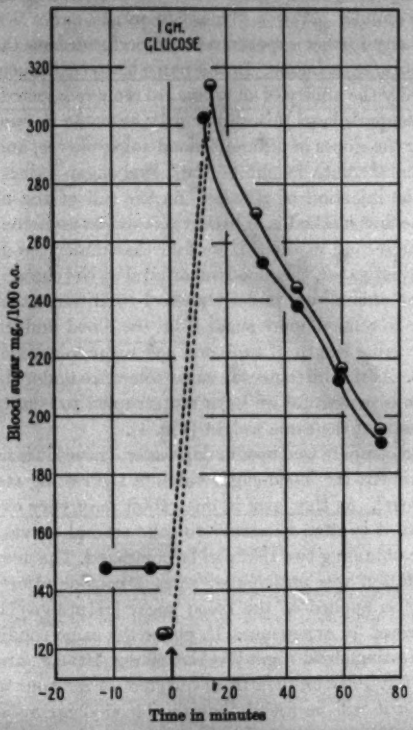

Fig. 2. Two glucose tolerance curves, after the injection of 1 g. of glucose intravenously, obtained on two different occasions from the same animal, whilst taking the fat diet.

sit quietly in the box but showed hurried respirations, retracted eyelids or intermittent struggling, was discarded as unsuitable for this work. Considerable hæmorrhage is required to produce an adverse effect upon the sugar-removing mechanism. If, the moment a blood sample is taken, an assistant stops the bleeding, there will be no error from this source, but

as a precaution it is advisable to reduce the number of samples to a minimum in experiments of considerable duration. Improvement in an animal's capacity to remove blood sugar may be found if a second experiment is performed too soon after one in which much glucose was given to the animal. After a glucose tolerance curve 6 weeks should elapse before any further experiments are performed on that animal.

Comparison of experiments. In this paper the term "glucose tolerance" is used to signify the ability of an animal to remove injected glucose from the blood. Comparison of this ability may be made in two ways, firstly by comparing the slopes of different blood-sugar curves, and secondly by comparing the absolute height of the blood-sugar values at standard times after the injection of glucose. As the fall of the blood sugar is hyperbolic the first method is of little value unless numerous samples are taken. By the second method, if we find that under the dietetic conditions being investigated, the blood-sugar level at the chosen time is lower than when the animal was under standard conditions, then the animal has been able to remove more sugar from the blood under the new conditions in the same length of time and the sugar tolerance is increased, and *vice versa*. As the differences in sugar tolerance under the influence of different diets is so definite we have not scrupled to compare curves on different animals of the same weight (Fig. 4).

In order to compare two insulin depression curves it is necessary that in each experiment the blood-sugar value in the resting state should be at the same level. As this level in one rabbit may vary over a range of 30 mg./100 c.c. it is often necessary to take several curves on the same animal before obtaining two that can be compared. The necessity for this tedious precaution was anticipated from a consideration of the mass action theory as applied to the blood sugar [Himsworth, 1931], and was often verified by experience. If, under the same conditions, on one occasion the resting blood-sugar level is 140 mg./100 c.c., and on a second occasion 115 mg./100 c.c., then three-quarters of an hour after one-half unit of insulin it will be found that the blood-sugar values will be at approximately the same level. It will appear that in the first experiment one-half unit of insulin had in the same period of time depressed the blood sugar 25 mg./100 c.c. more than in the second experiment. If, now, these two curves are charted so as to start from the same resting level the first experiment will appear to indicate a greater susceptibility of the animal to insulin (cf. Fig. 1). That this is not so will be revealed when, under the same dietetic conditions, two insulin depression curves are obtained which actually start from the same resting level. The curves are

then identical. It may be taken as a general rule that a standard dose of insulin will, in an animal on a fixed diet, cause a greater and more rapid fall of blood sugar when the resting blood sugar is high than when it is low. It will be seen that, ideally, the resting blood sugar values in curves to be compared should be at the same level (Fig. 5), but that if we are expecting the conditions under investigation to increase the effect of

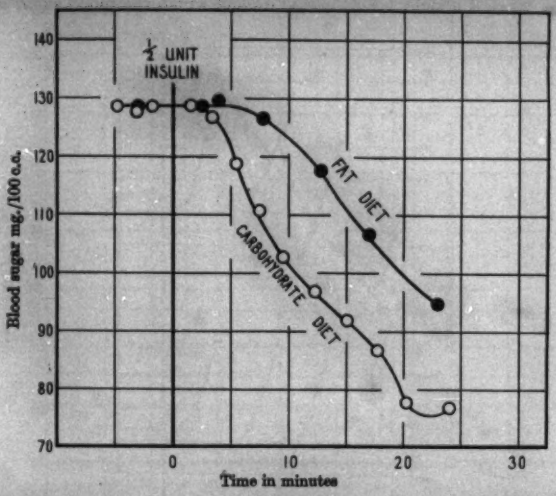

Fig. 3. Two insulin depression curves from the same animal, one (discs) obtained on the fat diet, the other (circles) obtained on the carbohydrate diet. The resting blood-sugar level on the fat diet was 134 mg./100 c.c. whilst on the carbohydrate diet it was 128 mg./100 c.c. Under the same dietetic conditions this difference in the resting levels would have tended to produce a more rapid and deeper fall in the curve commencing at 134 mg./100 c.c. As this tendency acts against the result expected, and obtained above, it has been considered allowable to compare these two curves despite their commencing at different resting levels.

insulin then if the resting blood sugar under these conditions is lower than under the standard conditions we may compare the curves, but if it is higher then we may not. Similarly if the new conditions are expected to decrease insulin effect we may only compare the curves if the resting blood sugar under these conditions is higher (Fig. 3). It is hardly necessary to add that only curves obtained from the same animal may be compared.

Results.

The figures show typical results from each group of experiments.

In Fig. 3 are shown two insulin depression curves obtained on the same animal, one whilst receiving a carbohydrate diet and the other whilst taking a fat diet. It will be observed that, after the injection of insulin, there is a short latent period during which the blood sugar is un-

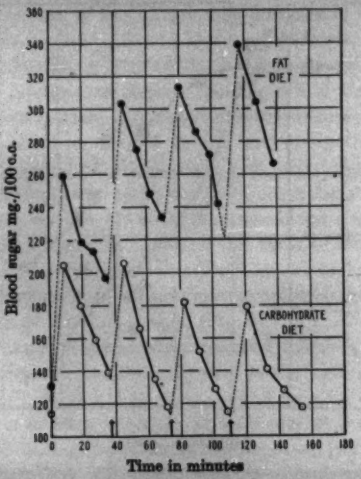

Fig. 4. Two consecutive glucose tolerance curves after injection of 1 g. of glucose every 35 min., the one (discs) obtained on the fat diet, the other (circles) obtained on the carbohydrate diet. The times of injection are indicated by the arrows.

affected, and that this period lasts for only 2 min. in the curve on the carbohydrate diet whilst it lasts for 6 min. in the curve on the fat diet. The significance of the latent period has been discussed elsewhere [Himsworth, 1933]. After this delay, depression of the blood sugar commences, and the experiments show that on the carbohydrate diet one-half unit of insulin exerts a more rapid and profound effect upon the blood sugar than when the animal is receiving a fat diet.

Fig. 4 demonstrates that the sugar tolerance is decreased by giving a fat diet. The experiments with four injections of glucose belong to the consecutive glucose tolerance group, but from comparison of the curves

after the first glucose injection in each experiment the diminished tolerance, on the fat diet, is obvious.

Fig. 5 shows that intravenous injection of glucose sensitizes the animal to the influence of insulin so that the same dose exerts a greater and more rapid effect upon the blood sugar after glucose than before. In

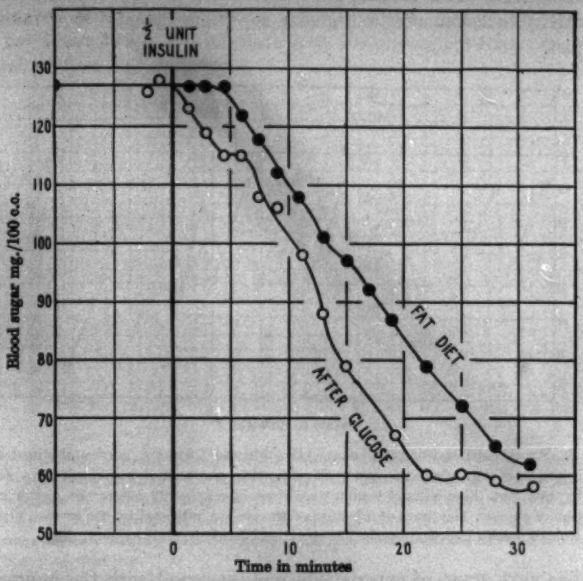

Fig. 5. Insulin depression curves, both obtained whilst the animal was on the fat diet, the one (discs) taken under the routine basal conditions, the other (circles) 40 min. after the blood sugar had fallen to rest after four injections of 1 g. of glucose at intervals of 35 min. The resting blood-sugar level in each case was 127 mg./100 c.c.

this experiment four injections of 1 g. glucose were given intravenously at intervals of 35 min. and then the blood sugar allowed to return to normal levels. Insulin was not injected until 40 min. after the blood sugar had returned and remained steady at the resting level. It was necessary to allow this period to elapse, otherwise it might, quite justifiably, have been maintained that the more rapid and profound effect of insulin after the glucose injections was due to a continuation of the fall of

the final sugar tolerance curve and not due to a true increased susceptibility of the animal to insulin. This experiment was carried out thirteen times with the same result on different animals. Six of the animals had been on a fat diet for over 3 months; the rest were on a carbohydrate diet. The difference between the two groups was not striking, but indicated that animals living on a carbohydrate diet showed a greater increase in sensitivity to insulin after four glucose injections than did those taking a fat diet.

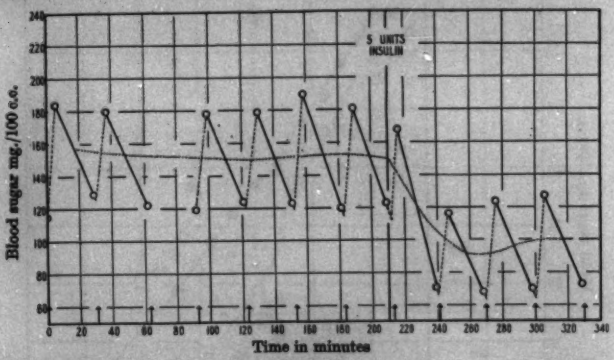

Fig. 6. Experiment showing the consecutive glucose tolerance curves obtained after injection of 1 g. of glucose every 30 min. when the animal was receiving a carbohydrate diet. Five units of insulin were given intravenously before the eighth injection of glucose. The times of injection of glucose are indicated by the arrows, and the time of insulin injection by the heavy vertical line.

The next group of experiments was concerned with the improving tolerance to carbohydrate that occurs after each successive dose of glucose, the so-called Staub-Traugott phenomenon. Figs. 4, 6, 7 and 8 show the effects of successive doses of glucose intravenously. The lower curve in Fig. 4, the first seven curves in Fig. 6, the first four curves in Fig. 7, and the first five curves of the lower experiment in Fig. 8 demonstrate this phenomenon. For convenience of visualization a curve has been drawn (dotted line) through the average figure of the two blood-sugar values for each curve at the time midway between the two samples. It will be seen in the curves mentioned that each subsequent blood-sugar curve begins and ends slightly lower than did the preceding one and that the "average curve" falls. Thus the phenomenon is present in rabbits

after intravenous injection and does not require that glucose be absorbed from the intestine. The writer has come to regard this effect as one of the most delicate phenomena in carbohydrate metabolism. In Fig. 7 the first four curves show an improving ability of the animal to deal with glucose. During that time the animal was perfectly quiet. As the fifth injection of glucose was about to be given the rabbit struggled violently in the box, and, as we discovered afterwards, injured its back. The disturbance did not last above 30 sec., but its effect upon the subsequent tolerance curve is evident.

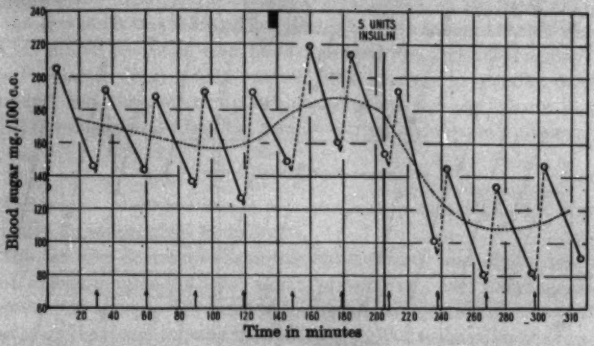

Fig. 7. Consecutive glucose tolerance curves obtained after injection of 1 g. of glucose every 30 min. when the animal was receiving a carbohydrate diet. Times of glucose injection indicated by arrows. During the fifth curve the animal struggled violently and the time of this occurrence is shown by the rectangle. Five units of insulin were given intravenously before the eighth injection and this time is marked on the chart by the heavy vertical line.

Now all animals in which this phenomenon of improving tolerance commenced after the first injection of glucose were receiving a carbohydrate diet. In animals on a fat diet the effect was either not observed or did not appear until several glucose injections had been given. Thus in the upper curves of Figs. 4 and 8 the blood sugar rises progressively higher after each injection of glucose. In the upper experiment shown in Fig. 8 there is no sign of improvement until after the sixth injection. This influence of diet is invariably the same. On a high fat diet not only does the tolerance not improve, but it is at first unequivocally worse after each injection, until finally after several injections a tardy improvement appears.

In the final group of experiments a comparison of the ability of a standard dose of insulin to suppress the hyperglycæmia after the intravenous injection of glucose was made between animals taking the two types of diet. These experiments were the logical sequel of those previously described, in which it was shown that the ability of insulin to

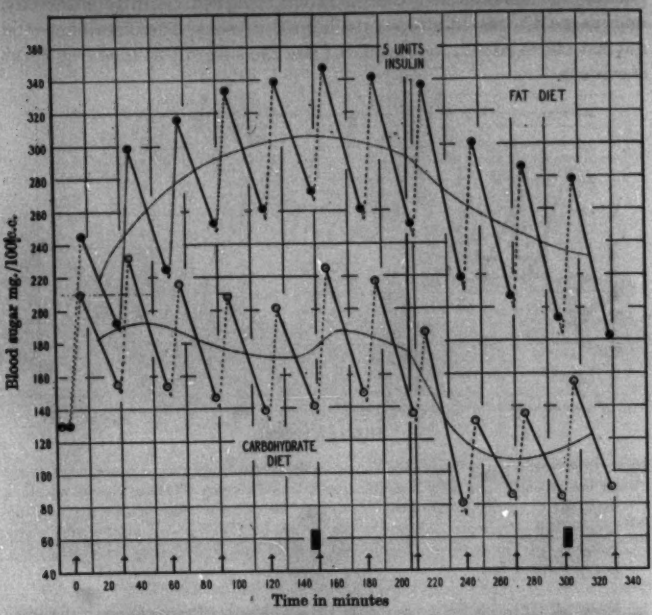

Fig. 8. Two series of consecutive glucose tolerance curves performed on the same animal after injection of 1 g. of glucose every 30 min. The upper curves (discs) were obtained when the animal was receiving a fat diet, the lower (circles) when taking a carbohydrate diet. For each experiment the arrows indicate the times of glucose injection and the heavy vertical lines the times when five units of insulin were given intravenously. The black rectangles indicate periods of struggling during the performance of the lower experiment.

depress the blood sugar was increased after intravenous injections of glucose. It was argued that if insulin were injected during the course of a consecutive glucose tolerance curve, then the increasing susceptibility of the animal to insulin consequent upon the preceding injection of glucose

would be revealed by an increasing ability to suppress hyperglycæmia. The dose of insulin injected intravenously was 5 units and it was preceded either by two, four or seven injections of 1 g. of glucose at intervals of 30 min. Four further glucose injections were given after the insulin and the tolerance curves obtained from these were compared with the curves obtained before insulin. In Figs. 6, 7 and 8 are shown the results of these experiments in which seven preceding injections were given. In Figs. 6 and 7 the animal was receiving a carbohydrate diet, and the marked effect of insulin in suppressing the rise of blood sugar is obvious. In Fig. 8 are shown two experiments of this type obtained on the same animal, the upper when it was taking a fat, the lower when taking a carbohydrate diet. It will be seen that when the experiment was performed during the period of fat feeding, insulin exerted little effect in suppressing hyperglycæmia, but that when the same experiment was carried out in the period of carbohydrate feeding, insulin exerted its effect in a pronounced degree.

Discussion.

The results presented in the previous section can at this point conveniently be summarized as follows.

The fat diet decreases sugar tolerance; retards and diminishes the action of insulin upon the blood sugar; prevents or delays the progressive improvement of sugar tolerance which occurs on injection of consecutive doses of glucose; and impairs the ability of insulin to diminish the hyperglycæmia following intravenous injection of glucose.

The carbohydrate diet improves sugar tolerance; accelerates and increases the depression of the blood sugar by insulin; favours the progressive improvement of sugar tolerance after consecutive injections of glucose; and does not impair the action of insulin in reducing the hyperglycæmia after intravenous administration of glucose.

Reference has been made to observations by previous authors on the effect of starvation upon the sugar tolerance curve and the depression of the blood sugar by insulin. It has been found that sugar tolerance is reduced, insulin action is diminished, and [Du Vigneaud and Karr, 1925] the ability of one dose of glucose to reduce the hyperglycæmia produced by a second is impaired. Thus it appears that starvation produces the same deleterious effect upon the ability of the body to deal with sugar as the giving of a diet with a high fat content.

Various theories have been put forward in explanation of the effects of starvation, administration of glucose, fat diets and carbohydrate diets upon the organism's sugar tolerance, but little attention has been paid

to the effects of the same factors upon insulin action. So far no attempt at correlating the two sets of observations appears to have been made. In the light of the correlation demonstrated by the above experiments the previous theories on these conditioned variations of sugar tolerance become untenable.

The previous theories are of two types, those which attribute the change in tolerance to variations in the reaction of the tissues, and those which ascribe the change to alteration in sensitivity of the insulin-secreting mechanism. Prior to 1925 the "tissue reaction theory" was put forward by workers whose observations had been concentrated on the effect of diet on sugar tolerance, whilst the "theory of varying insulin secretion" was held by those who had more particularly investigated the effect of consecutive doses of glucose upon the sugar tolerance. After this date the latter phenomenon was correlated with the effect of variations in diet, and at the present time the "theory of varying secretion" is the most generally quoted. It is evident, however, that the two hypotheses were not mutally exclusive.

The "tissue reaction theory" will be considered first.

Haldane, Wigglesworth and Woodrow [1924] showed that rapid and temporary changes in tissue reaction, either to the alkaline or to the acid side, influenced sugar tolerance adversely. Du Vigneaud and Karr [1925] give glucose tolerance curves from rabbits after starvation and 18 hours after administration of either a fat or carbohydrate meal. Starvation and a previous fat meal had the usual adverse and a carbohydrate meal the usual beneficial effect on the sugar tolerance. These authors claimed that the administration of sodium bicarbonate before the test neutralized the effect of starvation or ingestion of fat. Their further results, however, did not indicate that this effect was dependent upon change in the *p*H or alkali reserve of the blood. Since this time various authors have investigated this problem in man but the results have been conflicting. My own results on this point [Himsworth, 1933, complete results not yet published] showed that long-continued ingestion of large doses of ammonium chloride or sodium bicarbonate had no effect upon the sugar tolerance and resulted only in a reduction or augmentation of the alkali reserve, which was fully compensated, within the extremes of the normal range of *p*H by changes in the CO_2 tension of the blood. It was possible to show that, in man, the *p*H of the arterial blood remains at the normal level whether a high fat or a high carbohydrate diet is given, that if, on the high fat diet, sufficient alkali were taken daily for ten days to produce a persistent compensated alkalosis, the decreased sugar tolerance

still persisted unchanged, and that if, on the carbohydrate diet, sufficient ammonium chloride were given to produce a persistent compensated acidosis the high tolerance for sugar remained unchanged. It must, however, be understood that it is not suggested that these results exclude the possibility of some change in tolerance being produced by sudden and more extreme variations in *p*H consequent upon single massive doses of acid or alkali, nor that the taking of single large amounts of fat or carbohydrate by an organism adjusted to an ordinary diet may not produce a significant change of the *p*H. It is, however, believed that these results prove beyond doubt that the changes in sugar tolerance consequent on a high fat or a high carbohydrate diet in man are not due to changes in reaction of the tissues.

Hamman and Hirschman [1919], who first described the improvement in tolerance consequent upon ingestion of repeated doses of glucose, merely observed that the phenomenon was due to stimulation of the mechanism which removed glucose from the blood. Traugott [1922] ascribed the improvement to stimulation of liver function. In this year data on the effects of administration of insulin became available, and Foster [1922] attributed the greater tolerance seen after a second dose of glucose to the effect of insulin secreted in response to the first dose. Thalheimer *et al.* [1926] showed that continuous injection of glucose, at a uniform rate, resulted in a rise of blood sugar which was followed by a steady decline to normal or subnormal levels, and they also ascribed this result to the secretion of insulin consequent upon the continuous stimulation of the islet cells by the inflow of glucose. In 1927 Sweeney published his paper on the effects of starvation and of fat, protein and carbohydrate diets upon the sugar tolerance. He definitely regarded the beneficial effect of carbohydrate as being due to an increasing sensitization of the insulin-secreting mechanism to changes in the blood sugar, as a result of the repeated stimulus of excessive carbohydrate consumption, whilst the adverse effect of fat and of starvation he attributed to the desensitization of the same mechanism consequent on the absence of this stimulus. Macleod [1930] endorsed this explanation and said "...we may imagine that when there is abundance of pre-formed carbohydrate (free sugar and glycogen) entering the circulation, the islets of Langerhans are stimulated to produce larger amounts of insulin than usual, and to secrete it more abundantly, in response to temporary increases in blood sugar."

We shall now discuss this theory in the light of our results. In any of the consecutive glucose tolerance curves on the carbohydrate diet the

improvement in tolerance can be seen after each dose of glucose (Figs. 4, 6, 7). This improvement is slight, but when we compare these curves with those obtained on the fat diet (Figs. 4, 8), it is evident that the impressive thing is not that the improvement after glucose is small in degree, but that, after each glucose injection, a great decrease in tolerance does not occur. It is evident that to prevent such a decrease a mechanism of considerable power is brought into play more strongly on the carbohydrate than on the fat diet. In contrast with the results on a fat diet the improvement seen on the carbohydrate diet though slight is significant.

It has been shown that insulin exerts a greater effect on the blood sugar when it has been preceded by an injection of glucose. Glucose injection, therefore, results in the production of a state of increased susceptibility to insulin, *i.e.* each unit of insulin on injection has, actually or apparently, become more active. Zunz and la Barre [1927] have shown that hyperglycæmia is an adequate stimulus for the secretion of pancreatic insulin. If we now consider the phenomenon of improving tolerance after consecutive doses of glucose in animals on a carbohydrate diet (Figs. 4, 6, 7), we see that after the first dose of glucose at least two processes must occur. Insulin is secreted in response to the hyperglycæmia but, in addition, there is produced some degree of the state of increased susceptibility to insulin. These two factors combine to reduce the blood sugar. Let us suppose that the curve is allowed to return to rest at the original level. The experiments with insulin demonstrate that the susceptible state persists and, therefore, the pancreas must now be secreting less insulin than it did before glucose was given, otherwise, the animal being more susceptible, the blood sugar would sink and remain at levels considerably lower than the original. Neither in rabbits nor man does this occur. The second dose of glucose is now given. Insulin is again secreted in response to hyperglycæmia but, owing to the animal being more susceptible, each unit of insulin secreted now exerts a greater effect than in the preceding curve. Thus less insulin will be required to bring the blood sugar back to normal. But it has been found that the degree of susceptibility increases with each subsequent injection of glucose up to at least eight injections. If, after four injections, the blood sugar is allowed to return to rest, it will be found that it remains steady within a few mg. per 100 c.c. of the original level, and yet, by direct experiments, it has been demonstrated (Fig. 5) how much more susceptible the animal now is to insulin. The secretion of pancreatic insulin now required to maintain the blood sugar at this level must be very much less than was required in the pre-experimental state. These deductions indicate that, not only is the current

explanation of increasing insulin secretion after each consecutive injection of glucose untenable, but that any explanation which does not recognize that insulin is secreted in decreasing amounts after each dose of glucose is equally incompatible with experimental results. It would appear then that insulin plays a decreasingly important part in reducing the blood sugar in the consecutive glucose tolerance curves, and that in such experiments the improving tolerance, or absence of decreasing tolerance, is mainly due to an increase in the organism's susceptibility to insulin.

This conclusion is accentuated by the results of experiments in which consecutive tolerance curves were performed on animals taking a high fat diet (Figs. 4, 8). In the upper experiment of Fig. 8 it will be seen that first the tolerance decreases and then that an improvement sets in after the sixth injection of glucose. Just before the eighth injection five units of insulin were given intravenously. A very slight effect on the tolerance resulted. This result is strikingly different from the same experiment carried out on the same animal when receiving a carbohydrate diet (lower experiment, Fig. 8) (cf. also Figs. 6, 7). Whatever was lacking in the animal on the fat diet which the same animal on the carbohydrate diet possessed, it is evident that this was not insulin. The animal on the fat diet was practically insusceptible to insulin. It is worthy of note that almost certainly this result was not due to the presence of adrenaline. Fig. 7 shows the effect of an injury on the sugar tolerance. The injury was probably followed by secretion of adrenaline which caused the subsequent diminution in tolerance, yet in this experiment five units of insulin exerted its usual marked effect (cf. Fig. 7). We can only conclude that the impairment of sugar tolerance shown on the fat diet is due to the animal's diminished susceptibility to insulin and its impaired ability to develop this state in response to administration of glucose. This conclusion is fully endorsed by those experiments in which the action of insulin on the blood sugar was directly compared in the same animal under conditions of fat and of carbohydrate feeding (Fig. 3).

The idea derives further support from the results obtained by various authors when investigating the effects of starvation. Dann and Chambers [1930] have shown, in dogs, that after a 3 weeks' fast the administration of 50 g. of glucose results in hyperglycæmia and glycosuria; that in normal animals sugar causes a definite rise of R.Q., but that in the starving ones the same amount of glucose produces practically no change from the resting value. In an attempt to compensate for this suppression of the ability to oxidize carbohydrate, large doses of insulin

were given, to the starved animals, along with the first dose of glucose. A rise of R.Q. of normal magnitude was not obtained; even enormous doses of insulin could not restore the normal specific dynamic action of glucose. The missing factor was evidently not insulin. It was found, however, that administration of 50 g. of glucose on consecutive days produced a progressive improvement of the resulting sugar tolerance curve so that, on the fifth day, this had returned to normal. This improvement was paralleled by a gradually increasing respiratory response until, on the fifth day, 50 g. of glucose again produced the normal rise of R.Q. Thus, by exactly the same means that had been shown to increase the sugar tolerance and susceptibility to insulin, the normal metabolic response to glucose had been restored. It is of interest in this connection that Baur [1929] showed in men that, after 85 g. of glucose, the oxygen intake was higher after a carbohydrate rich diet than after a carbohydrate poor diet or fast. It appears justifiable to conclude that, in starvation, the decreased sugar tolerance, the impaired effect of insulin on the blood sugar, the small improvement in tolerance after successive doses of glucose, and the impairment of the specific dynamic action of glucose, are all dependent upon the inefficiency of one common mechanism, the mechanism which is responsible for the development of the state of susceptibility to insulin.

Our final conclusion, therefore, after a consideration of the effects of starvation, fat diets, glucose administration and carbohydrate diets is, that the varying ability of an animal to deal with carbohydrate characteristic of each of these conditions is not due to a variation in response of the insulin secreting mechanism, but is due to a change in the animal's susceptibility to insulin. The mechanism behind the development of this susceptible state remains unknown, but it would appear justifiable to suggest that it is probably intimately connected with insulin action. Two simple possibilities present themselves. The diminished susceptibility following starvation or the taking of a fat diet may be due to the production in excess under these conditions of an inhibitor of insulin. The increased susceptibility consequent upon the administration of glucose or carbohydrate diets may result from increased production of an activator of insulin, it being postulated that insulin is prepared and as secreted by the pancreas is an inactive precursor of that substance which, in the body, produces the effects we recognize as following injection of insulin. It will be seen that both possibilities may also co-exist, each exerting its influence on the phenomenon under observation in proportion to its concentration in the body at that time. In previous papers [Himsworth,

1932, 1933] other evidence has been brought forward in favour of the activation hypothesis, and it will be seen that all the experimental results recorded in this communication can be explained on the basis of an activation of insulin, an activation in which the speed is dependent upon the quantity of activator which has been transferred to the tissues in proportion to the stimulus of carbohydrate administration.

Summary.

1. Experiments are recorded showing:

(*a*) That the administration to a rabbit of a diet rich in fat decreases the sugar tolerance; retards and diminishes the action of insulin upon the blood sugar; prevents or delays the progressive improvement of sugar tolerance which occurs on injection of consecutive doses of glucose; and impairs the ability of insulin to diminish the hyperglycaemia following intravenous injection of glucose.

(*b*) That the administration of a diet rich in carbohydrate improves the sugar tolerance; accelerates and increases the depression of the blood sugar by insulin; favours the progressive improvement of sugar tolerance after consecutive injections of glucose; and does not impair the action of insulin in reducing the hyperglycæmia after intravenous administration of glucose.

2. From the results of other workers it is shown that starvation produces the same effect on glucose tolerance and insulin action that we have demonstrated as characterizing the high fat diet. The diminished specific dynamic action of glucose found after starvation and after a high fat régime is brought forward as another point of similarity.

3. The current theory that the diminished tolerance after starvation and the taking of a fatty diet is due to a diminished sensitivity of the insulin secreting mechanism, and that the improved tolerance after glucose injection and administration of a carbohydrate diet is due to increased sensitivity of the same mechanism, is shown to be incompatible with the observed results.

4. It is concluded that these results can be completely explained on the basis of the experimental observation that fat diets and starvation diminish the susceptibility of an animal to insulin, whilst carbohydrate diets and administration of glucose increase the susceptibility to insulin.

5. It is suggested that the susceptibility of an organism to insulin can be explained by the hypothesis that insulin as prepared and as secreted by the pancreas is an inactive substance, that it is activated in the body by an

unknown factor and that the production of this unknown activator is increased when carbohydrates are given, and diminished when they are withdrawn.

I should like to express my thanks to Prof. C. R. Harington for his help and criticism.

The expenses of this work were partially defrayed by a grant from the Government Grant Committee of the Royal Society, to whom my thanks are due.

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PINEALECTOMY IN RATS, WITH A CRITICAL SURVEY OF THE LITERATURE.

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(*Received December* 29, 1933.)

The occurrence of premature puberty in some cases of pineal tumours in the male has stimulated many attempts to find a functional relationship between the pineal gland and reproduction. The experiments involving the feeding of pineal or the administration of pineal extract have been, in the main, negative. The effect of castration, pregnancy, and other changes in the phases of reproduction on the pineal has been studied with conflicting results [Krabbe, 1923]. The same is true of the work on the effect of pinealectomy on reproduction. The conflicting evidence has led us to reinvestigate the problem. By avoiding at least some of the errors of earlier workers, we have arrived at a definite answer to the question as to whether pinealectomy affects reproduction.

Literature.

A survey of the results of extirpation of the pineal shows that about half of the authors obtained premature development of the sex organs, more often in the male than in the female, frequently accompanied by increased body growth, while the remaining workers obtained negative results. A critical review of the methods of data shows that all of the work is open to criticism on at least two points: (1) the normal range of the age of puberty and rate of growth of the particular breed under the specific laboratory conditions of the experiment has not been studied. (2) The necessity has not been recognized of using enough animals under standardized conditions of strain, age of operation, diet, living conditions, and age of death to allow of statistical analysis of results. This standard is difficult to apply because of the high mortality of the operation.

The species in which the effects of pineal ablation have been studied are the rat, guinea-pig, rabbit, dog, chick, and frog. The work will be reviewed by species.

(1) *Rat.*

Foà [1914] pinealectomized eight rats weighing 60–85 g. each, of which three males and one female survived. He found that the body growth and the development of the testis of the operated animals preceded that of the controls, although by 4 weeks after operation they were the same. However, his charts and description show that these changes were within the normal range. Horrax [1916] pinealectomized a series of rats, most of which died of an epidemic infection, and reported that the results on the four male survivors were inconclusive. Kolmer and Löwy [1922] removed the pineal by thermocautery from rats weighing 30–50 g. Rats of three litters survived and no changes were noted in them after 2 months.

Izawa in 1926 carried out a more complete and careful experiment, checking the completeness of the pinealectomy by serial sections of the operative site. He used twenty day-old albino rats, and killed them at the age of 85 days. He obtained data from a total of eleven male animals with complete pinealectomy, with six controls, five males with incomplete removal and three controls, ten females with complete removal and six controls, and four with incomplete removal and three controls. Izawa found that the pinealectomized animals grew better than the controls, and that some of the organs related to reproduction were significantly heavier in the operated animals. These organs were: in the female, the hypophysis, ovary, and uterus; in the male, the testis and epididymis; and the eyeballs in both sexes. This group of experiments constitutes the best evidence for a change in growth and reproduction following pinealectomy in mammals. Inspection of Izawa's figures, however, reveals several facts that weaken his conclusions.

(1) Izawa's operated animals were slightly heavier at the age of 20 days than the controls. Study of the weights which are taken weekly as a routine on all the rats of our colony shows that a slight difference between the weights of young litter mates at weaning is increased during the growth period, although after the age of 100 days the lighter animals often become as large as the others.

(2) The animals of each group vary widely in body weight. This is more striking in the controls. For example, three rats, each having the abnormally low weight of 76·1 g. (which seems an unusually uniform figure) at the age of 85 days, are included in the group of male controls. One

male control rat weighed 176 g. Why the supposedly normal rats should vary from 76·1 to 176 g., a difference of 130 p.c. of the weight of the lighter animal, is not explained. The pinealectomized rats varied from 105 to 206·5 g. in weight at the age of 85 days. It is obvious that there was lack of standardization in some way—infected animals were not eliminated, the food varied, or the size of litters was not standardized. None of these factors is mentioned.

(3) As a result of the wide variation of body weight, calculation of the probable error of the mean weight for the different groups at 85 days showed that the difference between the mean body weights of the control and operated animals is not statistically significant. The probable error of the mean by the formula P.E. $= \sqrt{\frac{\Sigma d^2}{N}}$ by our calculation from Izawa's data is as follows: male operated $168{\cdot}7 \pm 19{\cdot}4$ g.; male controls $136{\cdot}1 \pm 25$ g.; female operated $155{\cdot}2 \pm 11{\cdot}2$ g.; female controls $131{\cdot}9 \pm 9{\cdot}6$ g.

(4) It is common knowledge that retarded growth is accompanied by a delay in the appearance of puberty and in the growth of the reproductive organs. The fact that the operated rats had larger reproductive organs cannot be attributed to the operation unless their better body development can be so attributed. Since the effect of the operation on somatic growth is open to doubt, we may consider the point unproven. Izawa does not give data on the actual weights of the organs.

del Castillo [1928] removed the pineal from ten female rats at the age of 18 days by thermocautery. Two animals were operated on in a similar manner, except that the pineal was left intact and there were two normal controls. The growth curves and age of puberty (48–49th day) were similar in the operated and control animals. The absence of pineal tissue was demonstrated by serial sections.

Renton and Rusbridge [1933] pinealectomized eleven female and five male rats at about 26 days of age, using controls which had undergone a similar operation without the removal of the pineal, and also normal controls of the same litters. The rats were killed 340 days after operation. The absence of the pineal was verified histologically and the endocrine and reproductive organs were weighed. No significant changes were found. Accepting Izawa's work, the authors concluded that the changes which he noted were transitory.

(2) *Guinea-pig.*

Horrax [1916] removed the pineal from eighty-two guinea-pigs, and performed a craniotomy on sixty-two controls between the ages of 2 days and 7 weeks. Of these, forty-eight pinealectomized and forty-two control animals survived. The data were obtained from fifteen pairs of males and twenty pairs of females, each pair consisting of a pinealectomized and a control animal which were operated on and killed on the same day. The operation had no perceptible effect on the female guinea-pigs, except that the three operated animals which gave birth to litters did so at an interval of 3 weeks to 10 days before the first of the two controls which had litters. This slight difference is obviously not significant. The males were pinealectomized at various ages between the 7th and 49th day and killed between the 49th and 98th day, with a post-operative interval of 35–72 days. The mean body weight was approximately the same for the pinealectomized and control series, but the mean weights of the testes and seminal vesicles were greater for the operated animals. The mean weight of the testes was 854·3 mg. for the operated and 663·3 for the control groups. However, the wide scattering of the figures for the individual animals makes these mean figures insignificant: the testes of the operated animals varied from 140 to 930 mg. and of the controls from 170 to 1700 mg. The testis would obviously vary greatly in weight during the ages of 49 and 98 days, which includes the period of maximum growth of the body as well as of the testes, and a mean obtained for such a series is not significant.

We may therefore conclude that these experiments do not prove that pinealectomy produces increased or premature development of the testes. Clemente [1923] pinealectomized five guinea-pigs under the age of 1 month, of which two males and one female survived. He found the males more aggressive than the controls. After a temporary retardation in growth they became heavier than the controls and the volume of the testes was larger. The females were kept with the males, and the operated female when killed was found to have two fœtuses *in utero* in a more advanced stage of development than the single fœtus in the uterus of the control. These data are obviously an inadequate basis for his conclusion that pinealectomy produces precocious sexual development.

(3) *Rabbit.*

Exner and Boese [1910] removed the pineal from ninety-five rabbits with a thermocautery. Most of the animals died of intraventrical hæmorrhage within 12 hours, and of the remainder three males and three

females grew to maturity. All were fertile, and the females had normal litters. No abnormalities were noted. Foà [1912] removed the pineal from a series of young rabbits, none of which survived. Sarteschi [1913] operated on twenty-three animals at the age of 45 days, and of these three survived for some time. One male died of an infection at 5 months and had a larger testis than the control; the second, a male, was found to have a fragment of pineal and did not have an abnormally large testis; the third, a female, had a litter at 5 months.

Clemente [1923] pinealectomized a series of rabbits 1 month old, of which one, a male, survived. This was killed after 3 months, and changes similar to those found in his one completely pinealectomized guinea-pig were noted.

(4) *Dog.*

Sarteschi [1913] operated on fifteen dogs of which three survived. These grew rapidly and had sterile coitus at 6 months. They were killed at 9 months and no abnormalities were noted other than a large testis and adiposity. Sarteschi concluded that pinealectomy produced precocious sexual development, adiposity, and precocious growth. The breed and care of the animals were not described. Dandy [1915] operated on dogs (number not noted) at the ages of 10 days to 3 weeks. Two females lived to the age of 14 months and were in heat at the age of 1 year. Several males lived 3–8 months without any evidence of sexual precocity or retardation. The data were not reported in detail. Dandy concluded that pinealectomy had no observable effect on the development of his animals.

(5) *Chick.*

Foà [1912 *a*, *b*] was the first to remove the pineal in chickens with success. He used sixty-three chicks of the same strain between the ages of 3 and 5 weeks, and of these three males and twelve females survived. He performed a craniotomy on three male and twelve female controls of the same ages. In the males the growth, development and plumage were less good at first, but after 2–3 months the development was rapid. There was premature development of the secondary sex characters. Autopsy was performed 8–11 months after operation, at which time the testes and comb were much larger in the pinealectomized animals. There were no macroscopic differences in the other organs. The differences in age at first crowing, and at first mating, do not appear significantly different, but the weight of the testes varied from 11–15 g. in the controls and from 18–22 g. in the operated animals; and the weight of the comb was

41–50 g. in the controls and 72–88 in the operated animals at the age of 6–8 months. The females showed no changes. He repeated the experiment in 1914 on two male and five females with three male controls, and confirmed his previous findings. He sectioned the testes and states that there was proportional hypertrophy of the various parts. He again repeated the experiment in 1928, this time using ninety-three day-old chickens and killing the two operated animals at 367 and 555 days and the one control at 455 days. He measured the volume of the seminiferous tubules and interstitial tissue by means of serial sections and confirms his previous finding on the proportions of these tissues. In all of these experiments together there are data on seven pinealectomized cocks, killed at various ages from 200 to 555 days, with an equal number of controls not killed at the same ages as the operated animals. In each instance the testis of the operated animal is definitely larger than that of its control, but if the operated chicks reported in 1928 be compared with the controls of 1912, the testis weight will be found closely similar. There is a wide variation in the figures for testis and comb weight in both the operated and control series as assembled from the data from all these papers. Moreover, a careful comparison of the data in the two tables in the 1928 paper, all apparently based on the same three chickens, shows that there are inconsistencies in the figures: for example, the age at autopsy of no. 1 is given as 367 in one table and 267 in the other, while the testis weight in the control is 8·75 in one table and 10·75 in the other, and the body weights for cocks nos. 2 and 3 are reversed, all these errors occurring in the two tables in the same paper, published in 1928. The same inconsistencies are found in the corresponding tables published in the French translation of this paper, published in 1929. Zoia [1914] confirmed Foà's findings, but gave no details of his experiments. Christea [1912], quoted by Krabbe, removed the pineal from thirty young cockerels, of which twelve survived. These showed marked retardation in the development of the comb, spurs, crow, and plumage. The testes were atrophied, but the body growth was not much delayed.

Urechia and Grigorion [1922] reported that two cocks surviving pinealectomy showed no increase in testis weight over the controls, although the combs appeared slightly larger. The pituitary was three times as large in the controls. Histological studies revealed an increase in interstitial cells in the testis and of acidophilic cells in the hypophyses of the operated animals. Clemente [1923] obtained two surviving male chicks and confirmed Foà's findings. Izawa [1923] pinealectomized thirty-six chicks between the ages of 4 and 5 weeks, of which three males

and one female survived the complete removal. An equal number of controls of the same age were studied. He confirmed Foà's findings in regard to the male, but also noted precocious puberty in the female as shown by a heavier ovary and longer Fallopian tube as compared with one control of the same age. He studied various tissues histologically and found that the pinealectomized chicks showed: an increase in interstitial cells of the testis in the male; larger follicles in the ovary of the female; higher epithelium and an increase in colloid in the thyroid; and no changes in the adrenals, parathyroid, thymus, and pancreas. Badertscher [1924] removed the pineal from sixty-seven chicks at the age of 4 or 5 days. Of these, ten males and fourteen females survived to maturity and were killed when 228 or more days old. Histological examination of the site of operation revealed no traces of the pineal in five males and six females. The growth curves were similar in the completely pinealectomized, incompletely pinealectomized, and control birds of the same sex. The testes and combs showed approximately the same degree of development in the males killed at the same age, whether operated on or not. Badertscher concludes that his experiments indicate that pinealectomy does not affect the well-being of the chicks.

It is apparent that no one series of pinealectomized chicks is sufficiently large to be treated statistically. Badertscher is the only observer who considered this point, and it is probable that he is right in believing that the testicular hypertrophy reported by Foà and others and the atrophy noted by Christea show a misinterpretation of figures for testis weight, which are within the normal range of variation.

6. *Frog.*

Adler [1914] removed the pineal from 850 tadpoles which were 20–25 mm. long. Nine of these survived more than 2 weeks and all of these died before metamorphosis was complete, most of them with œdema of the skin. Hoskins and Hoskins [1919] were unable to remove the pineal completely and the tadpoles grew normally. Atwell [1921] found that pinealectomy in 8 mm. tadpoles did not alter the pigmentation. No conclusions as to the effect of the operation on reproduction can be drawn.

Method.

The rat was the animal of choice for several reasons: (1) we had abundant data on hand as to the age of puberty and the weight of the endocrine glands in a strain which we have bred and used for some years; (2) the pineal lies in a superficial position in the rat; (3) the young are

very immature when born; (4) because of large litters, it is easy to obtain litter-mate controls and sufficient animals to study statistically.

The animals were bred in the laboratory during the spring of 1933 and fed on McCollum B diet with the addition of yeast and lettuce. Twenty-four litters were used. The operation was performed when the rats were 1 day old in sixteen litters, and 2 or 3 days old in the remaining eight litters. The litters were limited to six on the day of operation. All runts were discarded. Ether anæsthesia was used. The instruments were sterilized in 80 p.c. alcohol, and sterile technique was used. There were occasional breaks in technique because of the difficulty of working with so small a field. The top of the head of the rat was washed with 80 p.c. alcohol, a mid-line incision was made, and a square skull flap was made by incising with a small lancet at a point over the convexity of the right lobe of the cerebellum, and extending the incision to the left about 1 mm. past the mid-line. The incision was then continued anteriorly from both right and left ends to a point about 3 mm. anterior to the transverse sinus. The dura was then separated from the skull and the square flap of skull lifted up and bent anteriorly. The dura was incised over the cerebrum just to the right of the pineal. The pineal was thus exposed and removed with the forceps. There was a little hæmorrhage, but this was usually easily controlled by light pressure with cotton. The first attempts at operation produced a high percentage of death by hæmorrhage because the transverse sinus was torn. This was avoided in most of the later cases. The bone flap was replaced and the skin sutured.

Two types of controls were used: a normal control and an "operated" control. In the latter the operation was performed in the manner above described, except that the pineal was left intact. An attempt was made to obtain an operated, a normal control and an operated control rat of the same sex from each litter, and this was possible in the majority of cases. The animals were then treated according to the routine in our colony. They were weaned at 28 days of age. Rats which died before the end of the experiment were autopsied to reveal the cause of death, but the organs were not weighed. In the females the age and weight at which the vagina opened was noted and the vaginal smear was observed daily until death. The female rats were killed between the ages of 86 and 90 days. All of the females of a litter were killed on the same day. The body weight, body length, vaginal smear, size of uterus, and the presence of infection at the site of operation or elsewhere were noted. The pituitary, thyroid, adrenal, and thymus were carefully dissected and weighed to

0·1 mg. in a closed weighing bottle. These organs and the uterus and ovaries were then fixed in Zenker.

The males were killed in two groups, at the ages of 60 and 70 days. All of one litter were killed on the same day. These ages were chosen because the normal age of puberty in the male rats of our colony was found in an earlier experiment to be about 60 days. The autopsy data included: body weight, body length, examination of saline suspension of sperm from the tail of the epididymis, a notation as to the development of the prostate and seminal vesicles, and the weight of the endocrine organs as in the female, with the addition of the weight of the testes. The site of operation, the middle ears and lungs were inspected for evidences of infection.

The brains of all the pinealectomized rats of both sexes were fixed in formalin, and in the operated animals the pineal area was serially sectioned and searched for remnants of pineal tissue. Small fragments of the stalk were found in three instances.

Observations.

(1) *Operative mortality.*

In all twenty-four litters were used. After limiting each litter to six and discarding from the experiment the controls whose operated litter mates of the same sex had died, there remained 125 rats. The statistics as to operative mortality are given in Table I, and the expected improve-

Table I. Statistical summary of the survival of the 125 rats used in the experiment.

		No. of rats			Mortality p.c.
		Survived	Died	Total	
Complete pinealectomy	1st–12 litters	13	20	33	60
	2nd–12 litters	17	8	25	32
	Total	30	28	58	49
Operated controls	1st–12 litters	12	4	16	25
	2nd–12 litters	11	10	21	49
	Total	23	14	37	40
Controls		28	2	30	6

ment in the percentage survival after experience with the operation is evident. The chief cause of death was infection of the operative site. In the first four litters several animals died at operation from hæmorrhage, and the number was not recorded. After that time forty-four animals

died. Of these eleven, including two controls, died within the first ten days after the operation, and most of them had either been mutilated by the mother or were so autolysed when found that the probable diagnosis of hæmorrhage was confirmed in only a few cases; of the remainder, thirty-two died of infection of the operative wound and one died of internal hydrocephalus without gross evidence of infection. This emphasizes

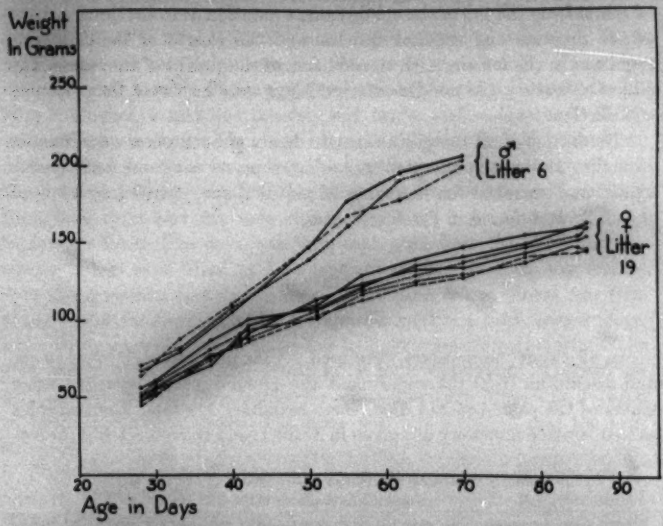

Fig. 1. Growth curves of pinealectomized and control rats. Operation at 2 days in litter no. 6 and at 1 day in litter no. 19. No infections were found at autopsy except for a slight infection in the female operated control. The vagina opened at 41 days in all the rats of litter no. 19.

—— = Operated. ..... = Normal. – – – – = Operated control.

the difficulty as well as the necessity of sterile technique in brain operations on young rats.

(2) *Growth.*

The animals were weighed weekly after weaning and no changes were noted in the growth curves of the operated animals. The final weights were also similar (Tables IV, V and Fig. 1).

(3) *Puberty in the Female.*

The age and weight at the opening of the vagina are given in Table II. All of the animals in which ear or lung infections were found at autopsy

TABLE II. The age and weight at puberty in the female pinealectomized and control rats as shown by the opening of the vagina.

	Operated or control	No.	Age		Weight	
			Mean	Range	Mean	Range
Uninfected	Operated	6	41	41–43	92	85–105
	Operated controls	4	44	40–46	99	90–106
	Normal controls	7	44	41–47	96	85–106
Infected	Operated	8	51	40–65	98	85–123
	Operated controls	8	44	36–57	92	96–109
	Normal controls	6	48	39–57	102	82–117

are listed separately. Previous experience has shown that some of these have delayed puberty, but it is not possible to estimate whether a given degree of infection will produce changes in growth and reproduction.

(4) *Puberty in the male.*

Spermatozoa were found in the tail of the epididymis in all animals killed at 60 days, but in some instances these spermatozoa were scanty or non-motile, or both, which is the case in animals killed a week or two before puberty [Andersen, 1932]. In these animals the seminal vesicles were small. In all instances of immaturity according to this criterion the middle ears, lungs, or both were found to be infected. The score is approximately the same for the operated and control animals (Table III).

TABLE III. The age of puberty in the male as shown by the presence of mature motile sperm in the tail of the epididymis.

	Age 60 days		Age 70 days	
	Mature	Immature	Mature	Immature
Operated	4	3	8	1
Operated controls	5	1	4	1
Normal controls	5	3	7	0

The infections were sufficient to slow the growth in most instances without other external changes. Our data therefore show that our uninfected rats, whether pinealectomized or not, mature by the 60th day, but there is not proof that the pinealectomized animals did not mature earlier. However, there is no significant difference in the development of the secondary sex organs or in the testis weights by the 60th day, and therefore it is not probable that the pinealectomized animals matured earlier.

TABLE IV. The weight of the endocrine glands in pinealectomized male rats.

Age killed days	Op., op. control or normal	Infected	No. of rats	Body weight in g.	Body length in mm.	Weight in mg.			Weight in g.		Mg./kg. body weight			Weight in g./kg.	
						Pituitary	Thyroid	Adrenal	Thymus	Testes	Pituitary	Thyroid	Adrenal	Thymus	Testes
60	Op.	No	2	178	190	6·7	26·4	26·1	0·330	2·48	37·5	150·2	147·5	1·86	14·06
60	Op. control	No	0	—	—	—	—	—	—	—	—	—	—	—	—
60	Normal	No	4	169±8	185±5	7·2±0·4	25·4±2·3	24·7±2·2	0·411±0·035	2·29±0·07	42·4±1·9	151·7±16·0	148·0±18·0	2·27±0·10	13·6±0·5
60	Op.	Yes	5	144±14	174±6	5·7±0·5	17·1±3·4	24·3±2·3	0·351±0·062	1·90±0·24	39·4±2·7	116·4±16·4	171·3±23·2	2·20±0·23	13·1±1·0
60	Op. control	Yes	6	162±17	180±6	6·9±1·0	22·3±4·6	27·2±3·2	0·375±0·062	2·30±0·26	42·2±2·8	135·2±15·6	170·0±20·4	2·45±0·24	13·6±0·7
60	Normal	Yes	4	137±20	175±6	6·3±0·6	16·5±5·6	28·4±5·6	0·264±0·093	1·98±0·16	47·5±7·0	114·6±21·8	227·9±76·2	1·85±0·59	14·0±1·5
70	Op.	No	6	184±20	190±6	6·5±0·8	23·3±5·0	28·5±3·1	0·322±0·016	2·16±0·38	35·3±1·7	132·4±10·2	157·8±19·4	1·80±0·22	11·9±1·5
70	Op. control	No	4	199±3	196±3	7·2±0·3	24·7±0·6	26·4±1·3	0·384±0·053	2·55±0·21	35·5±2·3	121·0±33·2	129·0± 5·2	1·88±0·25	12·3±1·2
70	Normal	No	5	194±3	193±3	7·4±0·4	26·3±2·6	27·6±1·6	0·390±0·043	2·56±0·22	38·6±1·7	135·7±12·8	142·1± 6·6	2·01±0·21	13·2±1·0
70	Op.	Yes	3	177±16	186±6	6·3±0·8	25·2±2·6	22·8±1·1	0·351±0·032	2·41±0·17	35·4±1·6	142·2±4·1	130·5±10·1	2·04±0·08	13·8±1·0
70	Op. control	Yes	1	150	180	5·4	20·4	26·9	0·268	1·28	36·0	136·0	179·2	1·78	8·6
70	Normal	Yes	2	189	193	6·2	19·1	27·0	0·351	2·56	34·6	100·5	142·9	1·86	13·6

TABLE V. The weight of the endocrine glands in pinealectomized female rats.

Killed at 85–90 days.

Op., op. control or normal	Infected	No. of rats	No. with regular cycle	Body weight in g.	Body length in mm.	Weight in mg.			Weight in g.	Weight in mg.			Weight in g.
						Pituitary	Thyroid	Adrenal	Thymus	Pituitary	Thyroid	Adrenal	Thymus
Op.	No	6	5	155±2	183±3	9·4±0·9	21·3±3·3	39·7±3·4	0·271±0·009	60·7±5·8	137·7±20·8	257·0±22·1	1·76±0·32
Op. control	No	4	4	159±5	185±3	11·4±0·4	24·2±1·3	42·8±5·5	0·282±0·030	72·1±4·4	148·6± 6·3	268·0±28·0	1·78±0·20
Normal	No	7	5	157±6	185±2	9·9±1·1	20·6±1·5	39·2±2·6	0·288±0·016	63·2±7·3	131·4±10·8	249·8±15·1	1·82±0·23
Op.	Yes	8	6	151±7	181±3	8·6±1·0	18·1±1·9	38·7±2·1	0·264±0·044	57·0±5·7	120·2±13·0	257·8±19·4	1·74±0·14
Op. control	Yes	8	7	157±8	183±2	9·3±0·7	19·8±2·0	37·9±3·4	0·278±0·019	59·4±5·3	118·7± 8·0	228·3±10·3	1·78±0·16
Normal	Yes	6	5	162±8	184±3	9·9±1·1	18·2±1·3	37·9±4·0	0·288±0·059	60·8±5·3	113·1±10·2	235·6±27·2	1·76±0·17

(5) *Endocrine glands.*

No significant changes were noted in the endocrine weights. The variations in the figures are greater in the females than in the males for the reason that the animals were killed at various stages of the œstrous cycle, and also that the cycles were irregular in some cases. The effect of irregular cycles and of the phase of the cycle has been discussed in previous papers [Andersen and Kennedy, 1932; Andersen, 1933]. The greater pituitary and adrenal weights in the group of operated control animals without infection may be safely attributed to the fact that all four of these rats had regular cycles and three were killed on the day after œstrus. The probable error of the mean weight of each organ for each group of rats was calculated by the formula $\text{P.E.} = 2/3\sqrt{\frac{\Sigma d^2}{N}}$. This is reported as an indication of uniformity of our figures. The series is admittedly too small for the probable error to be calculated with accuracy.

Discussion.

Pinealectomy is followed by so high a rate of operative mortality that no worker has obtained a series of pinealectomized animals large enough for statistical analysis of the data. The conditions of the many reported experiments have rarely been standardized and controls have been inadequate in most cases. The diverse reports as to the effect of the operation on puberty—that it produces premature or retarded sex development or does not affect it—have left the question open. We have attempted to settle the problem by eliminating the errors which we perceived in the work of our predecessors. By using very young rats, standardizing the breed, diet, and age at death, eliminating rats in which a fragment of pineal remained and grouping the uninfected rats apart from those having incidental infections, we have been able to obtain groups of pinealectomized, operated control, and normal control rats of both sexes, which we believe to be comparable. There is very little difference in these groups in either sex in the rate of body growth, age at puberty, or development of the reproductive and endocrine organs.

Conclusions.

Complete removal of the pineal gland of the rat between the ages of 1–3 days does not influence the rate of growth, age of puberty, or the weight of the pituitary, thyroid, adrenal, or thymus in either sex; it does not affect the weight of the testes in the male, or the occurrence of normal œstrous cycles in the female.

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THE OXYTOCIC PROPERTY OF THE BLOOD OF THE COW.

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(*Received January* 8, 1934.)

There have been a few indications that there is an increase of oxytocic power—possibly due to posterior pituitary hormone—in the blood just before parturition. In 1918 Cow showed that adrenaline causes a relaxation of a non-pregnant guinea-pig uterine horn, an action which previous treatment with infundibulin reverses. Heller and Holtz [1932], however, found that adrenaline normally causes contraction of the guinea-pig uterus in late pregnancy. They accordingly suggest that in late pregnancy the uterus is under the influence of infundibulin circulating in the mother's blood. Küstner and Biehle [1927] found that blood obtained from pregnant women, and especially from women in labour, enhances the power of pituitrin to cause expansion of the frog's melanophores, whereas blood from non-pregnant women or from women in the puerperium actually inhibits slightly this action of pituitrin. Fontes [1930, 1931] claimed that the serum from a parturient woman is markedly oxytocic. This oxytocic property disappears 6 hours after parturition. Jeffcoate [1932] failed to confirm this work. Morris [1933] showed that there is, several days before parturition in goats, a great increase in nitrogen and urinary output. This is followed, immediately *ante partum*, by a decrease in nitrogen excretion. This disturbance of nitrogen metabolism can be imitated by injections of "pituitrin."

The present work is an attempt to follow this problem further. The cow was used as the experimental animal since large quantities of blood can be safely withdrawn.

Method.

The excised guinea-pig uterus was used to estimate oxytocic potency. As there are fallacies connected with addition of protein to the bath containing the uterus it seemed essential to produce a protein-free extract.

Maclean [1928] dialysed blood through membranes against Ringer solution. Unfortunately this method dilutes the oxytocic substance and moreover is slow. Anselmino, Hoffmann and Kennedy [1932] filtered blood through collodion membranes. This method involves technical difficulties and is again slow. The method adopted in the present series of experiments was as follows: blood was taken from the left external jugular vein and run into 5 p.c. sodium citrate (5 c.c. citrate per 150 c.c. blood). This was centrifuged to get rid of the red cells, the plasma was decanted and 1 c.c. of concentrated hydrochloric acid (36·6 p.c. HCl) was added to every 10 c.c. of plasma. The acid was added slowly, the flask being rotated to prevent the formation of a thick gelatinous mass. The precipitated protein was removed by means of the centrifuge. The supernatant fluid was decanted, raised to the boiling point, and quickly cooled. This precipitated any protein remaining suspended in the acid solution. After centrifuging once more the supernatant fluid was decanted off. This acid extract gave none of the usual tests for protein (precipitation by heavy metals) but gave a faint biuret reaction. This may have been due to metaproteins or peptides. Since, however, the conditions of preparation were kept rigidly constant it was not to be expected that this would affect the estimations. The clear solution was kept on ice until required, and was neutralized, just before addition to the bath, with a 10 p.c. solution of sodium hydroxide, usually about 0·4 c.c. being required for each 1 c.c. of acid extract. The volume of the test solution was nearly the same as that of the original blood.

100 c.c. bovine blood yields 60 c.c. plasma [Blackwood, 1932].
6 c.c. conc. HCl are added.
26·4 c.c. 10 p.c. NaOH are required for neutralization.
Final volume 92·4 c.c. test solution.
For calculation of results it was assumed that the test solution was equal in volume to the blood withdrawn from the vein.

In the actual estimations the method of Burn and Dale [1922] was followed, but, owing to the fact that we could expect only small quantities of oxytocic substance, we could not use submaximal doses. In preference we used the region $\frac{1}{4}$ to $\frac{3}{4}$ of full contraction as it has greater differential sensitivity [W. Storm v. Leeuwen, 1923; Sawasaki, 1925]. The reaction of the Burn and Dale fluid was always adjusted to *p*H 7·4, and oxygen with 5 p.c. carbon dioxide was used for aeration. In many cases it was found that the sensitivity of the preparation increased with each addition of blood extract. This was useful when extracts with very small oxytocic value were being examined. In the results the potency of the

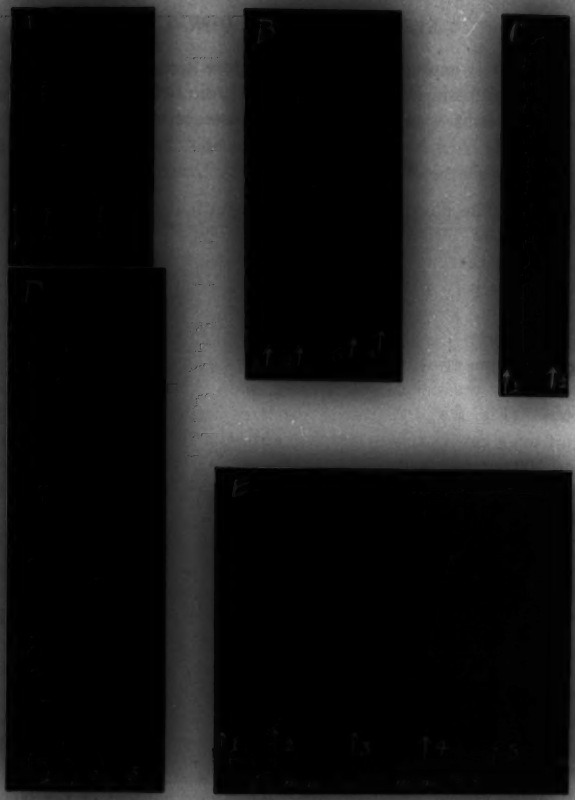

Fig. 1. Test solutions T prepared in the manner described in text. S, standard solution of pituitrin (20 o.u. per litre).

A. "Spring Morn," 14 days *ante partum*: (1) 0·7 c.c. S, (2) 5·0 c.c. T, (3) Repetition of 1.

B. "Kirkhill May Mist," 3 hours before calving: (1) 1·0 c.c. S, (2) 2·0 c.c. T, (3) Repetition of 1, (4) 1·5 c.c. T.

C. "McMinn," 22 hours *post partum*. (1) 5·0 c.c. T, (2) 0·3 c.c. S.

D and E. "Threave May Mist," 5 months pregnant. T_1 prepared in usual way. T_2 pituitrin added to blood to give 20 o.u. per litre. Solution then prepared in usual way.

D. Immediately after preparation of solutions. (1) 0·5 c.c. S, (2) 0·1 c.c. T_1, (3) Repetition of 1.

E. 24 hours later. (1) 1·0 c.c. S, (2) 0·2 c.c. T_1, (3) 0·4 c.c. T_1, (4) Repetition of (1), (5) 1·0 c.c. T_2.

blood extract is expressed in terms of international oxytocic units per litre, using pituitrin (Parke, Davis & Co.), diluted to give 20 oxytocic units (o.u.) per litre, as the standard of reference.

Results.

Heifers. The blood from fourteen virgin heifers was examined. In ten cases there was no sign of oxytocic power—less than 1 o.u. per litre. In four cases the oxytocic value varied between 1·0 and 3·5 o.u. per litre. The period of heat seemed to have no effect on the oxytocic power.

Cows. Forty-five blood samples were taken from fourteen cows and from two pregnant heifers. Dry and non-pregnant cows (Fig. 2 (1)), and cows in early pregnancy and in milk, showed no measurable oxytocic substance (Fig. 1 A). There was one exception—two separate samples taken from a cow in the fifth month of pregnancy had the equivalent of 40 and 100 o.u. per litre (Fig. 1 D). No other animal at any time showed oxytocic values approaching this, so that it must be regarded as exceptional. We first noticed measurable quantities—1–18 o.u. per litre—at the time of the "drop" (relaxation of the sacrosciatic ligaments). This occurs about 1 week *ante partum* in the cow but may occur 2–3 weeks *ante partum* in the heifer. This oxytocic value was maintained and increased up to the time of parturition (Fig. 1 B). In four cases there was a temporary drop in oxytocic content 2 or 3 days before calving. We do not know whether this is of significance, although it coincides in time with a fall in nitrogen excretion noted by Morris [1933].

Calved cows. We had six samples from normal cows in the first week of the puerperium—from 7 hours to 8 days after calving; none of these showed measurable oxytocic power (Fig. 1 C).

It would appear that normally the oxytocic content of the blood is very low (less than 1 o.u. per litre). During the last week of pregnancy the amount becomes measurable (*circa* 5 o.u. per litre). After calving the oxytocic value falls quickly to less than 1 o.u. per litre.

The nature of the oxytocic substance.

(*a*) Experiments *in vitro*. Pituitrin (Parke, Davis & Co.) was added to cows' blood. After removing protein in the usual way there was no loss, within the limits of experimental error, of the added pituitrin (Fig. 1 E (5)). This shows that pituitrin out of the ampoule survives the acidifying and boiling.

Citrated blood stored in the ice chest retains its oxytocic power, if present, for at least 24 hours. The acid solution prepared from the blood, however, does not retain its oxytocic power for 24 hours, even when stored in the ice chest. The acid solution prepared from blood to which pituitrin has been added, on the other hand, does retain its oxytocic

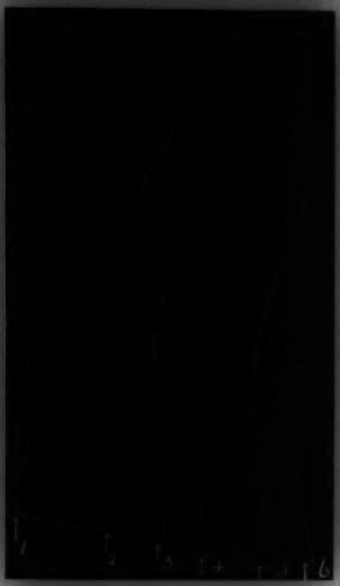

Fig. 2. "Hopeful." Test solutions *T* prepared as described in text. (1) 3·0 c.c. *T* from blood withdrawn immediately before injection of 10 c.c. pituitrin. (2) 0·7 c.c. *T* from blood withdrawn 1¼ hours after injection. (3) 0·05 c.c. standard solution of pituitrin (20 o.u. per litre). (4) 0·4 c.c. same *T* as in 2. (5) Repetition of 3. (6) 1·0 c.c. *T* from blood withdrawn 3¾ hours after injection.

power. The oxytocic substance is therefore much more labile than pituitrin from the ampoule (Fig. 1 D and E).

Certain other substances are capable of causing a contraction of the uterus, *e.g.* histamine. But it was soon found that on allowing the alkalinized blood extract to stand at room temperature for a few hours all its oxytocic property disappeared [Burn, 1928].

Van Dyke, Bailey and Bucy [1929], using the excised uterus method, state that the oxytocic substance of cerebrospinal fluid appears to be calcium. Friedman and Friedman [1933], using the rabbit uterine fistula technique, are in agreement. In the present series the greatest oxytocic values were found in late pregnancy and in milk fever where it is known that the serum calcium is lower than normal. Also the fact that this oxytocic effect disappears overnight is against the idea that calcium is the cause of the uterine contraction.

The test solution used contained 4·7 p.c. NaCl. The hypertonicity does not appear to induce contractions of the uterus, since there was never parallelism between the amount of test solution added and the height of contraction obtained (Fig. 2, 1 and 4).

(*b*) Experiments *in vivo*. Pituitrin was given intramuscularly on three occasions. Non-pregnant cows giving only a small amount of milk were used. In all three cases the oxytocic power of the blood was enhanced (Fig. 2), but to different degrees. This may be due to varying rates of absorption and/or varying rates of destruction and of excretion. The oxytocic substance recovered was labile in the same fashion as the oxytocic property of the blood from parturient cows.

Injected pituitrin causes therefore an increase in the amount of labile oxytocic substance of the blood. This increase is of short duration, suggesting that this substance is either destroyed or excreted very quickly. Dale [1909] suggests that injected pituitrin is excreted in the urine.

Summary.

With a few exceptions the oxytocic power of cows' blood is low; during the last week of pregnancy the oxytocic power increases. Immediately after parturition the oxytocic power falls to a low value. The oxytocic substance of blood is more labile than pituitrin, but is neither histamine nor calcium; its amount is increased for a short time by intramuscular injection of pituitrin.

We desire to express our thanks to Dr N. C. Wright for his continued interest in this investigation, and to Dr R. C. Garry for much valuable advice. We are especially indebted to Mr A. B. Fowler, who gave us great assistance with the taking of samples and with the obstetrical histories.

The cost of this research was borne mainly by the Hannah Dairy Research Institute, Ayr, but a small part of the expenses was defrayed by the D. C. Andrew Fund of the Institute of Physiology, Glasgow University.

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THE EFFECT OF LIGATION OF POSTERIOR CORONARY VESSELS ON THE ELECTRO-CARDIOGRAM IN EXPERIMENTAL BUNDLE-BRANCH BLOCK.

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(*Received November* 15, 1933.)

It has been reported by Averbuck and Rachmilewitz [1931] that in dogs in which the branches descending from the circumflex ramus of the left coronary artery on the posterior surface of the heart had been ligated, section of the right branch of the His bundle no longer gave electrocardiograms typical of bundle-branch block. It is of note that an anus-œsophagus lead alone was employed, and that although section of the branch was performed sometimes before, sometimes after tying the vessels, the electrocardiographic observations were always made within a few minutes or at most an hour of the ligation. The authors discussed their results, which they admitted to be surprising, at some length, and came to the conclusion that the absence of the typical picture of branch block was due to interference with the blood supply of a part of the specific conducting tissue in the left ventricle, brought about by the coronary ligatures, and that their curves were comparable to those obtained by Rothberger and Winterberg [1917] after section of the right branch and posterior division of the left branch of the His bundle.

During the course of an experimental study in which the writer was associated, carried out by Wilson and his co-workers on the results of coronary ligation in the dog, the opportunity was taken to repeat the section of the right branch in dogs with various infarcts [Wilson, Johnston, Hill and Grout, 1933*a*, 1933*c*]. The section was made always after an interval of 36–48 hours from the time of ligature, and fairly

typical bundle-branch block curves were obtained in both leads I and III. In particular, a dog in which the circumflex artery had been ligated near its origin 48 hours before, and which had in consequence a large infarct of the posterior wall of the heart, yielded the curves reproduced in Fig. 1. For permission to reproduce these curves, and to report this experiment, hitherto unpublished, I am indebted to Dr F. N. Wilson, of the University of Michigan. The curves in Fig. 1*a*, taken before interference with the right branch, show the abnormalities of *QRS* and *T* waves which are associated with an infarct in this situation, and which we need not here further discuss [see reference Wilson, Johnston, Hill

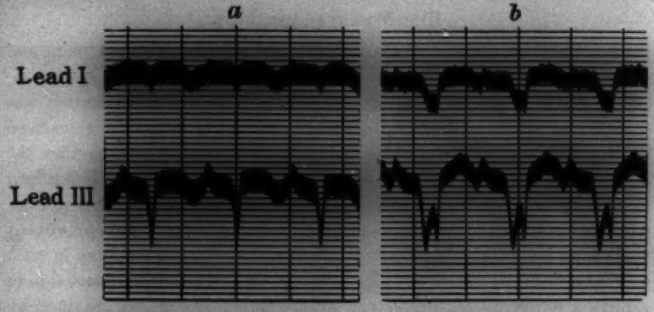

Fig. 1. Electrocardiograms of dog, simultaneously recorded in leads I and III. Standardization, 1 cm. per millivolt. Time marker indicates 0·2 sec. *a*, records 48 hours after ligation of circumflex branch of left coronary artery. *b*, after cutting right branch of His bundle.

and Grout, 1933*b*]. Section of the right branch of the His bundle produced (Fig. 1*b*) very marked broadening of the initial deflections, with the concordant biphasic type of complexes characteristic for canine bundle-branch block. There are minor abnormalities, such as the very marked splintering of the initial deflections in lead III, which is unusual in the dog though the rule in corresponding human curves, and the absence of any small initial *R* wave in either lead I or III; but the general form of the curves is typical enough.

This experience rendered untenable the explanation of Averbuck and Rachmilewitz for their results, for if in any dog ligation of the descending branches of the circumflex could compromise the blood supply of the specific tissue in the left ventricle, a dog with high circumflex ligation and very extensive destruction of the whole territory nourished

by that vessel should suffer from similar lesions of the conducting tissue; and of such lesions here there is no evidence.

In the course of discussion of the matter with Prof. C. J. Rothberger, in whose laboratory the observations of Averbuck and Rachmilewitz had been made, it was decided that the matter might profitably be further investigated, with a view to finding the explanation for two such widely divergent experimental results on the same subject. The experiments here reported, with the exception of that quoted above, were carried out to this end in Prof. Rothberger's laboratory. To him I am indebted for the facilities afforded, and for much advice and help.

Experimental methods.

For the experiments dogs were used, anæsthetized with morphine and chloralose. To avoid the troublesome arrhythmia common after morphine, both vagi were divided in all cases. Artificial respiration was maintained by a simple bellows and motor, connected to a tracheal cannula. This was provided with a side tube and stopcock, so that the air could be shut off during the taking of records, the lungs being held in the inflated position. This avoided the variations in the electrocardiogram which depend on changes in the lung volume and consequent variations in the electrical contact between heart and parietes in such a preparation. As the records lasted only a few seconds, changes due to asphyxia naturally play no part. Access to the heart was obtained by resection of the sternum and the adjacent portions of ribs and costal cartilages; the pericardium was then opened and stitched back, and the preparation was ready for such ligations, etc., as might be desired.

For the electrocardiograms two leads were used in all cases: transverse (lead I), and axial (anus-œsophagus, *A-O*). For the latter the customary German silver anal and œsophageal bougies were used; for the former, lead plates were sewed between the skin and chest wall on either side of the thorax. A condenser of 24 microfarads capacity was used in series with the string, to obviate the frequent adjustments necessary with the change of lead, which was effected by a simple Pohl's commutator.

Experiments were carried out on nine dogs; on some of these an attempted ligature of the ramus septalis had already been performed, but in no case was a dog used in which gross changes in the electrocardiogram were present, due to previous interference, and in half the dogs no complicating factors of this kind were present. Post-mortem examination of the heart in each case showed that section of the right bundle branch had been effectively performed.

Experimental results.

In several animals section of the right branch of the His bundle was carried out a few minutes after ligation of the posterior descending arteries. In all those similar results were obtained, which are illustrated in Fig. 2. In the animal from which these were derived, septal ligation had been performed one hour before the records shown in Fig. 2*a* were taken. There was depression of the *ST* segment in both leads I and *A-O* such as characteristically follows ligation of the ramus septalis, but signs of disturbed intraventricular conduction were absent. Fig. 2*b* is a record

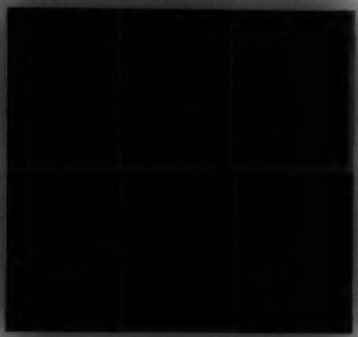

Fig. 2. Electrocardiograms of dog, by leads I and *A-O*. Time marker in these and succeeding records, 50 per sec. *a*, before posterior coronary ligation. *b*, 10 min. after ligation of posterior descending vessels. *c*, after cutting right branch of His bundle.

taken 10 min. after ligation of the posterior descending vessels. Lead I showed exaggeration of the *ST* depression previously present, and lead *A-O* a complex deformity of the ventricular deflections. In this lead there was no longer simple depression of *ST*, but elevation of that part of the electrocardiogram into a sinuous curve, which took origin from the descending limb of *R* and completely obliterated *S*. Section of the right branch (Fig. 2*c*) produced in lead I a ventricular complex of which *QRS* was typical for bundle-branch block, although the persistent depression of *ST* tended to deform the typical diphasic appearance of the complex taken as a whole. In lead *A-O*, on the contrary, the complex was quite atypical for bundle-branch block. *QRS* was of long duration (0·07 sec.), but was of low amplitude, was polyphasic and lay entirely

above the isoelectric line. *T* was high, rounded and positive. A very similar series of curves was obtained from another animal in which the procedures were exactly as in the experiment described above, but no septal ligation or other complicating feature was present.

In several experiments the order of procedure was reversed, and section of the right branch of the bundle was performed before the ligation of the posterior descending vessels. In those instances also the ventricular complexes in lead I remained fairly typical for bundle-branch block, while those in the axial lead altered considerably and ceased to be

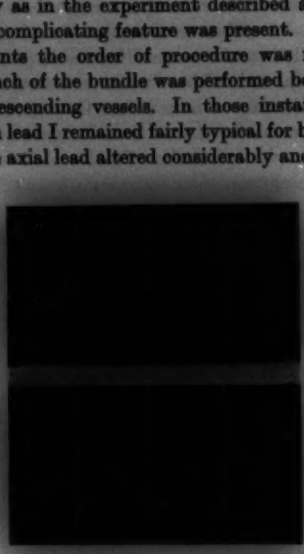

Fig. 3. Electrocardiograms of dog, by leads I and *A-O*. *a*, control. *b*, immediately after burning posterior surface of heart. *c*, after cutting right branch of His bundle, less than 2 min. after burn. *d*, 13 min. later.

typical in outline. The deformation of these complexes set in about 30 sec. after the tying of the vessels and progressed rapidly, so that in 2 min. they were no longer recognisable as those of bundle-branch block. The final type of complex closely resembled those previously obtained with section following ligation.

In three animals the effect upon the complexes in bundle-branch block of injury to the posterior surface of the heart was investigated. In the first of these, control curves had the form shown in Fig. 3*a*. The posterior surface of the ventricles was then cauterized superficially with a hot copper rod, care being taken to avoid all large vessels in case of intravascular thrombosis occurring as a complicating factor. The injury resulted in the type of *ST* deformity usual with posterior injuries, elevation

of that segment in the axial and depression in the transverse leads being produced (Fig. 3*b*). While these changes were still present, less than 2 min. after the burn, the right branch of the His bundle was cut. The resulting complexes are shown in Fig. 3*c*: it will be noted that in lead I a typical bundle-branch block curve was obtained, while in the anus-œsophagus lead a deformed complex, very similar to that obtained after posterior ligation, appeared. Fig. 3*d*, taken 13 min. later, showed the return of typical bundle-branch block curves in both leads on subsidence of the injury current; in lead I the only change is the disappearance of the slight depression of *ST* detectable in the previous curve, but in lead *A-O* the disappearance of the elevation of *ST* is associated with a deepening of *S* which restores the characteristic form to the ventricular complex in that lead.

Curves like those illustrated in Fig. 3 were obtained from another experiment of the same type, in which the right bundle branch had been cut, where subsidence of the injury current 9 min. after the cauterization was again attended by the appearance of typical bundle-branch block curves in the axial lead.

In two dogs occlusion of the vessels under investigation was performed after branch section in such a way that release of the obstruction could be made at any desired moment. This was effected by including a fine pledgelet of cotton wool in the ligature under the knot, rendering snipping safe and easy, or by simply occluding the vessel with a spring clip after it had been dissected clear and lifted from its bed. Fig. 4*a* shows curves from such an animal, where the posterior descending vessels had been occluded in the manner described for a period of 3 min.: the deformity of lead *A-O* is striking and of the type regularly found after such coronary occlusion. Fig. 4*b*, taken 2 min. later, shows even more bizarre deformity, still confined to the axial lead. On release of the ligature the complexes returned to the typical bundle-branch block form in a very short space of time. Fig. 4*c*, taken 1 min. after the release of the ligature, shows elevation of the later portion of *ST*, mere traces of the gross deformity present in earlier records.

Subsequent occlusion of the anterior descending ramus of the left coronary artery resulted in the curves shown in Fig. 4*d*, where the axial lead is practically unaltered, while the transverse lead (lead I) is grossly deformed after the same fashion as the *A-O* lead with posterior ligation. These changes in lead I developed in the course of a few minutes after occlusion of the vessel, and on release of the obstruction they too disappeared in a matter of seconds.

In another dog in which the right branch had been cut and the posterior descending arteries clamped, release of the obstructions was followed in less than half a minute by disappearance of the deformities of the *ST* segments. Occlusion of the anterior descending ramus in turn produced curves exactly comparable to those figured in the last experiment, and here again release of the ligature was followed by very rapid restoration of undeformed complexes.

Fig. 4. Electrocardiograms of dog, by leads I and *A-O*. *a*, 3 min. after occlusion of posterior descending vessels. *b*, after 5 min. occlusion. *c*, 1 min. after release of ligature. *d*, 3 min. after occlusion of anterior descending ramus.

Interpretation of results.

The first experiment performed suggested that the interpretation placed upon their results by Averbuck and Rachmilewitz could not be correct. Cutting the right branch of the bundle of His in this animal after ligation of the posterior vessels produced in the anus-œsophagus lead complexes very similar to those described by Averbuck and Rachmilewitz: but at the same time the complexes in lead I were practically typical for canine bundle-branch block. Such minor differences as did exist affected only the end deflection in that lead, and seemed to be

parallel to the deformations of the end deflection present before the cut was made—to be due, in fact, to the injury currents caused by the various ligations performed. This observation, that the complexes in the transverse lead were, so far as *QRS* was concerned, typical, even when those in the anus-œsophagus lead was quite unlike those usually obtained after section of the right branch, ruled out of court the explanation offered by the previous authors for their results. A lesion of the posterior division of the left bundle branch modifies not only the *QRS* in the axial lead, but also that of the transverse lead [Rothberger and Winterberg, 1917]. When, as here, the *QRS* of lead I remains typical for right bundle-branch block, then the changes in the *QRS* of the axial lead are probably due to some cause other than such a lesion of the left posterior division.

The observation of Wilson, Johnston, Hill and Grout [1933*c*] that 48 hours after circumflex ligation typical curves were obtained in both leads I and III on section of the right branch, appears also conclusive evidence that infarction of the specific tissue is not the cause of the observed abnormalities in the acute experiments under consideration. When the transient nature of the deformation is taken into account, the fact that it is present in acute experiments but absent after 48 hours, and when one remembers that the gross deformities of the *T* wave and *ST* segment following coronary ligation follow a similar time course, it seems probable that there is here more than a coincidental relationship. The later experiments were conducted with a view to determine whether a causal relationship existed or not.

Ligation of the posterior descending vessels in the otherwise normal dog heart produces depression of the *ST* segment in lead I, and much more pronounced changes in the anus-œsophagus lead of the opposite type, that is, elevation of *ST*, with, in the course of a few minutes, high take-off of *T* from the descending limb of *R*, and high, sharply convex, positive *T* wave. It is noteworthy that the changes are very much more marked as a rule in the axial than in the transverse lead. The curves obtained after bundle-branch section in such an animal are also as we have seen much more deformed in the anus-œsophagus lead than in lead I, and the deformity is confined to the later portions of the complexes (*S*, *ST* and *T*). In both leads it is in the same sense as the deformity produced by simple posterior ligation without right branch section.

On the other hand, anterior descending ligation in the dog produces changes which are more marked in lead I than in the axial lead, and which are the converse of those obtained by posterior ligation. Thus anterior descending ligation without further interference produces an

elevated *RT* segment in lead *I*, with high take-off of *T*, and "*T*-endôme," while in lead *A-O* a moderate depression of the *ST* segment results. If now in a dog we combine such a ligature with section of the right branch of the His bundle we obtain in lead *A-O* complexes that are typical enough, while in lead I the complexes are deformed in much the same manner as those in the axial lead after posterior ligation (see Fig. 4*d*).

In those experiments in which the ligatures were released when the electrocardiographic changes they had produced were at a maximum, it was remarkable that in a matter of 15–30 sec. from the restoration of the circulation the gross deformities of the ventricular complexes had completely disappeared. The rapid onset of the changes on occlusion of the vessels, and the even speedier disappearance on their release, speak strongly against implication of the conducting tissue in the ventricle as playing any part in the electrocardiographic changes. In contrast to the specific tissues of the sinus node and auriculo-ventricular node, which react promptly to oxygen lack [Lewis, White and Meakins, 1913; Rothberger and Scherf, 1927], it is an experimental fact that the conducting tissues in the ventricles are peculiarly resistant to anoxæmia [Pick, 1924; Tamazaki, 1930; Wachstein, 1932]. The contrast is exemplified by the speed with which the sinus node reacts to ligation of its vessels, becoming paralysed in a few seconds (described by Rothberger and Scherf, 1927, and confirmed in an experiment of this series), as compared with the long latent period before the bundle or its branches cease to conduct impulses after ligation of the septal artery [Kahn, 1911; Lauterback, 1928]. Purkinje tissue *in vitro* also will contract in oxygen-free perfusion fluid [Wachstein, 1932], or in the presence of cyanide in concentrations enough to abolish tissue oxidation in other tissues [Pick, 1924; Tamazaki, 1930].

Injury currents and *ST* deviation can be produced not only by coronary ligature, but by any manipulation that damages a considerable portion of the heart's surface. Such injury currents, produced, for example, by superficial burns, develop immediately on the infliction of the injury, and subside rapidly, in contrast to those produced by coronary ligation, which develop over a period of minutes and subside over a period of hours. The effect of such burns inflicted on one or other surface of the heart on the complexes in bundle-branch block was investigated in some experiments in this series and curves from two of these are reproduced. In these cauterization of a large area of the posterior surface produced pronounced elevation of the *RT* segment in the anus-œsophagus lead,

with high take-off of the *T* wave. The changes in lead I were, as with posterior ligation, less conspicuous, a slight depression of the *ST* segment being alone noted. Immediate section of the right branch of the His bundle produced curves in lead I and lead *A-O* in every way comparable to those obtained after a ligature of the posterior vessels and similar branch section. Curves taken about 10 min. later, at a time when the injury current produced in this way is known to be lessening or disappearing, were typical for bundle-branch block in both leads. The close dependence of the deformity on the injury current was thus established, and its independence of injury to the specific conducting tissues demonstrated.

Summary.

1. It has been claimed that in the dog, ligature of the vessels supplying the posterior surface of the left ventricle prevents the appearance of typical bundle-branch block on section of the right branch of the bundle of His. In the experiments cited, observations were made in all cases shortly after the ligature was tied, and an axial (anus-œsophagus) lead alone was employed. The explanation offered was that the ramifications of the conducting system in the left ventricle were compromised by interference with their blood supply. On the other hand it has been demonstrated that, in the presence of a gross posterior infarct, 48 hours after circumflex ligation in the dog, section of the right branch of the His bundle produced typical ventricular complexes in leads I and III.

2. The present investigation confirms the deformity of the ventricular deflections in the axial lead after right branch section, when observations are made soon after ligation of these posterior vessels. But lead I is at this time typical for bundle-branch block.

3. The results of ligation of the anterior descending artery, and especially the transient similar deformity in the axial lead produced by superficial cauterization of the posterior surface, suggest that the explanation does not lie in damage to the conducting tissues, but in the presence of an injury current due to acute infarction. After 48 hours this injury current has so far disappeared that when the right branch is divided at this time the curves obtained are undistorted.

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612.313.3:612.018

THE HUMORAL CONTROL OF THE SECRETION BY THE SUBMAXILLARY GLAND OF THE CAT FOLLOWING CHORDA STIMULATION.

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(Received December 29, 1933.)

As a corollary to the humoral theory one must presume that, no matter what method be used to stimulate the secretory nerve to a gland, the penultimate stage in the process of glandular excitation is the production of a chemical substance which in turn acts as the exciting agent to the gland cells. Many workers have shown that as a result of the stimulation of parasympathetic nerves a substance, apparently acetylcholine, is set free. Dale and Gaddum [1930] concluded that the contractures produced in the dog's tongue on stimulation of the chorda-lingual nerve could be explained most readily as being due to the liberation of acetylcholine. Bain [1932] stimulated the lingual nerve during perfusion of the dog's tongue *in situ*, and found that the perfusate contained a substance, apparently acetylcholine, produced in the process of vaso-dilatation.

Demoor [1913] collected saliva from a dog after electrical stimulation of the chorda and injected the saliva into a second dog. The injected animal responded with a secretion of saliva. The result of this experiment is difficult to explain except on the assumption that the saliva, produced by nerve stimulation, contained some substance capable of exciting secretory activity in the salivary glands. It is well known that one of the results of administration of acetylcholine is the secretion of saliva.

The present investigation was planned to test for the presence of acetylcholine in saliva and to investigate the possibility of liberation of this substance at the terminals of the chorda in the cells of the submaxillary gland of the cat.

Since completing the experiments described, my attention has been drawn to some recent publications on the subject.

Babkin, Gibbs and Wolff [1932] found that stimulation of the chorda in cats treated with physostigmine led to a fall in blood-pressure not wholly due to vaso-dilatation.

Gibbs and Szeloczey [1932], using the method of intermittent perfusion of the submaxillary glands of cats and dogs, found that the perfusate during chorda stimulation contained a substance resembling acetylcholine in its actions on the isolated frog's heart, on the rabbit's intestine, and on the blood-pressure of the cat. The substance "Chordastoff" is destroyed in blood except in the presence of physostigmine.

Babkin, Alley and Stavraky [1932] found that stimulation of the chorda on one side increased the secretion from the denervated submaxillary gland of the opposite side. Henderson and Roepke [1933] perfused the submaxillary gland in the dog and found that on stimulation of the chorda, the perfusate contained a substance which, on addition to the isolated frog's heart, resulted in diminished contractility, and if the concentration was high, in arrest of the heart. The active principle was destroyed by hydrolysis with alkali and the effect on the heart overcome by atropine. The authors concluded that the substance present was acetylcholine. Henderson and Roepke also found that after administration of physostigmine (0·25 mg./kg.) to dogs, stimulation of the vagus led to increased salivary flow from the denervated gland. Their attempts to show the presence of acetylcholine in saliva after stimulation of the chorda were completely unsuccessful.

Method.

Eighteen cats were used in the investigation. The cats were anæsthetized with sodium diethylbarbiturate, 0·4 g./kg. intraperitoneally. After an interval of 30 min. complete anæsthesia was obtained by giving ether, which could be discontinued after 15 min. The saliva was collected from a glass capillary cannula in Wharton's duct on one side. The lingual nerve on the same side was tied and cut proximal to the exit of the chorda-tympani and secretion evoked by faradization of the chorda-lingual nerve, the position of the secondary coil being constant in each experiment. In certain experiments the salivary flow was excited by intravenous injection of pilocarpine nitrate, a dose of 2·5 mg. being given to a cat of 3 kg. body weight. Salivary flow was recorded by means of a Condon's drop recorder. A record of the changes in the level of the carotid blood-pressure was taken. Intravenous injections were given through a cannula in the external jugular vein, the artery and vein used being contralateral to the gland under observation.

The collected saliva was diluted with 0·9 p.c. solution of NaCl, 0·5 c.c. saliva being made up to 3 c.c. before injection, and this was the dose usually given. Generally the saliva was injected within 10 min. of being secreted, but on several occasions it was allowed to stand at room temperature for 30 min. without any apparent diminution in its power.

In certain experiments, in order to inhibit the esterase present in the blood [Matthes, 1930], 0·2 mg. eserine salicylate per kg. was given.

In the experiments to test the nature of the substance present in the saliva, use was made of the findings of Dale [1914] that the vaso-dilator action of acetylcholine can be abolished by warming with alkalis or by previous administration of atropine. Accordingly, in the tests 0·5 c.c. saliva was heated in a boiling water bath with 0·5 c.c. *N* NaOH, then neutralized with *N* HCl and diluted to 3 c.c. with normal saline solution. It was found necessary to heat for 10 min. before this treatment became effective. After the effects of the hydrolysed saliva had been studied, the animals were given atropine sulphate, 2 mg./kg., and 5–10 min. later 0·5 c.c. saliva diluted but otherwise untreated.

As a further test of the nature of the active substance, in three experiments, after obtaining saliva by nerve stimulation or injection of pilocarpine, a portion of the animal's small intestine was suspended in a bath of 100 c.c. of Ringer-Locke solution at 36° C. After steady contraction rhythm had been attained, 0·5 c.c. of undiluted saliva was added to the bath and the effect on the plain muscle recorded. The Ringer-Locke solution was renewed, and the test repeated using saliva after hydrolysis with alkali.

In two experiments the cats were given an intravenous injection of acetylcholine bromide, 0·25 mg. to a cat of 3·5 kg., and the saliva resulting was diluted and injected into the animal.

Results.

1. *Intravenous injection of saliva obtained by stimulation of the chorda.*

Fig. 1 is typical of the effect on the vascular mechanism of injection of saliva. The tracing shows an immediate evanescent pressor effect followed by a marked depression of the blood-pressure, without any appreciable change in the heart rate, then a gradual return to the previous level. In some of the experiments the preliminary pressor effect was more marked than in the record shown, *e.g.* Fig. 8. The injection of saliva, 0·5 c.c., did not result in any secretion by the gland; 1 c.c. of saliva also failed to produce any secretion.

Fig. 1. The effect of intravenous injection of 0·5 c.c. autogenous saliva in normal saline solution into a cat at *A*. B.P. in mm. Hg. Time in this and subsequent records in 2 sec. intervals.

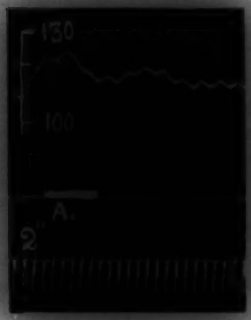

Fig. 2.

Fig. 3.

Fig. 2. Intravenous injection at *A* of 0·5 c.c. of saliva after hydrolysis with alkali and dilution.

Fig. 3. Intravenous injection at *A* of 0·5 c.c. saliva in saline solution after previous administration of atropine.

2. *The nature of the exciting substance in saliva.*

The form of the pressure curve in Fig. 1 is suggestive of that obtained on intravenous injection of small doses of acetylcholine [cf. Dale and Dudley, 1929], and to test this possibility saliva after hydrolysis with alkali was injected. Fig. 2 is a record of one such experiment. The pressor effect is slightly more prolonged but is not followed by the usual depression. In the earlier tests when the heating was not of sufficient duration

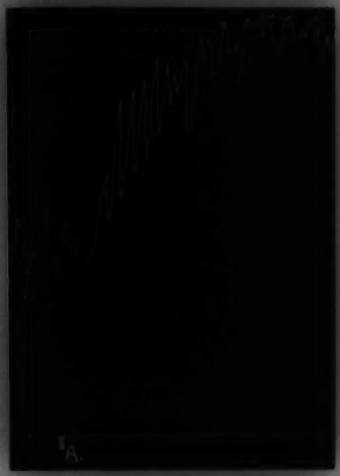

Fig. 4. Effect of 0·5 c.c. of autogenous saliva, added at *A* to 100 c.c. Ringer-Locke solution, on the contractions of the small intestine.

it was common to get a slight depression, but in every case the extent of the fall in the level of the blood-pressure was much less than that following injection of untreated saliva. Fig. 3 shows the effect of injection of saliva after atropine. The pressor effect is accentuated and the depressor effect abolished. Fig. 4 is a record of the effect of autogenous saliva on the plain muscle of the small intestine. The record shows the marked increase in tone resulting from the addition of 0·5 c.c. of saliva. Changing the Ringer-Locke solution resulted in relaxation of the muscle and within 5 min. the rhythmic contractions had returned. The addition of saliva after hydrolysis with alkali gave the result shown in Fig. 5. This record

shows that the exciting substance has been destroyed by hydrolysis. There is a period of relaxation of the muscle, after which the rhythmic contractions go on as before the addition of the saliva. The record shown in Fig. 4 is not identical with tracings obtained on addition of acetylcholine bromide to the bath under similar conditions. Acetylcholine causes a more abrupt contraction of the muscle, and the contraction is not maintained at the same level, but falls quickly to a lower level and then more slowly.

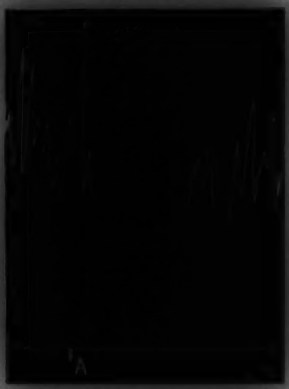

Fig. 5. Effect of 0·5 c.c. of autogenous saliva after hydrolysis with alkali, added at *A* to 100 c.c. Ringer-Locke solution, on the contractions of the small intestine.

An attempt is being made to investigate this difference in response to acetylcholine bromide and to saliva, with the object of using this method to determine the quantity of the active substances present in saliva secreted in response to different stimuli.

3. *Intravenous injection of acetylcholine bromide.*

The results of the preceding experiments suggest that the active substance in the saliva is acetylcholine, or some substance having identical biological effects, and that this substance is able to pass out from the gland in its secretion. In order to test this possibility acetylcholine bromide was administered with a marked depressor effect on the blood-pressure and an accompanying secretion of saliva. The saliva from acetylcholine stimulation was injected into the animal and the resultant effect

on the blood-pressure is recorded in Fig. 6. In the original injection of acetylcholine bromide the substance would be broadcast over the entire animal, only a small portion reaching the gland under observation, and if acetylcholine is the exciting agent for secretion this small amount has excited the gland and passed out in the saliva as shown by the result recorded in Fig. 6. The remainder of the dose of the acetylcholine would be destroyed by the esterase in the blood.

Fig. 6. Effect on blood-pressure of intravenous injection at *A* of 0·5 c.c. saliva (diluted) resulting from acetylcholine stimulation.

4. *Eserine and salivary acetylcholine.*

(*a*) *Nerve stimulation after eserine.* On stimulation of the chorda-lingual nerve a common, but not constant feature, was a slight, temporary fall of 10–15 mm. Hg in the level of the arterial blood-pressure. This is probably the result of local vascular change produced as the result of acetylcholine liberated at the terminals of the vaso-dilator fibres [Bain, 1932]. This vascular change is followed after a few seconds by a flow of saliva.

The possibility of the secretory activity being a result of the vascular change in the gland has to be considered, and it is possible that the sialagogue is liberated in the vessel walls. If this suggestion be correct any factor which increases secretion must do so by producing an increased

vascular change. Electrical stimulation of the chorda-lingual nerve after eserine resulted in a great increase in the rate and quantity of salivary flow; this increase in secretion was not accompanied by a corresponding increase in the vascular change. There was no secretion in response to the eserine injected, nor was there any prolongation of secretion after the stimulus was removed. Secretion ceased within 2 min. of removal of the stimulus. The results of chorda stimulation before and after eserine seem to suggest that the chief site of action of the chorda is on the gland cells and that the activating substance is not distributed to the secretory cells through the blood stream. If acetylcholine were liberated in direct contact with blood vessels, and only produced secretion after it had been carried some distance by the blood, one would expect protection of the

Fig. 7. Effect on salivary secretion (*S*) and blood-pressure of intravenous injection at *A* of 2 c.c. saliva in saline solution into cat after eserine.

acetylcholine by eserine and the resulting greater secretion but that this would be accompanied by a correspondingly increased effect on the blood-pressure level.

(*b*) *Injection of saliva and salivary secretion.* If saliva contains acetylcholine, as the results obtained suggest, and acetylcholine be the mediator between the nerve impulse and the gland cell, it is to be expected that injection of saliva will excite the gland to secrete. As stated above injection of 1 c.c. of saliva failed to excite secretion, but after eserine 1 c.c. of saliva caused a very small flow. Fig 7 shows the effect on the blood-pressure and salivary flow of injection of 2 c.c. of saliva into a cat 15 min. after eserine. In the earlier experiments intravenous injection of saliva produced marked vascular changes but no secretion of saliva, this lack of secretion is probably due to utilization (or destruction) of the acetylcholine before it reached the submaxillary gland.

After eserine protection had been given, the acetylcholine present in 1 c.c. of saliva was sufficient to give the secretory response. Obviously the fraction of the total acetylcholine, in the saliva injected, which will be distributed to the gland must be very small. Attempts at the determination of the actual amount of the active substance in the saliva necessary to evoke secretion have not yet yielded sufficiently reliable results to justify publication.

Fig. 8. Intravenous injection at *A* of 0·5 c.c. saliva (diluted) resulting from pilocarpine stimulation. *S*, salivary flow persisting from administration of pilocarpine nitrate.

5. *Pilocarpine stimulation of the gland.*

Pilocarpine on intravenous injection gives a picture of general stimulation of the parasympathetics with the resulting secretion of saliva. It was thought advisable to test the saliva produced by this method of stimulation for the presence of acetylcholine. Saliva secreted as a result of administration of pilocarpine nitrate was injected. The tracings resembled those obtained from injection of saliva resulting from electrical stimulation of the chorda (Fig. 8). The possibility of this result being due to leakage of pilocarpine into the saliva seems unlikely as the amount

present could only be a very small fraction of the dose given, and yet the effect on the blood-pressure is almost as great as that produced by the full dose of pilocarpine. Tests were made on the "pilocarpine" saliva similar to those made on "nerve" saliva. The effect of injection of "pilocarpine" saliva after hydrolysis with alkali is shown in Fig. 9. Here, as in the test on "nerve" saliva, the preliminary pressor effect is prolonged and the depressor effect abolished.

Fig. 9.

Fig. 10.

Fig. 9. Intravenous injection at *A* of 0·5 c.c. "pilocarpine" saliva after hydrolysis and dilution.

Fig. 10. Intravenous injection at *A* of 0·5 c.c. "pilocarpine" saliva (diluted) after previous administration of atropine.

Injection of "pilocarpine" saliva after atropine is striking (Fig. 10) and is sufficient to disprove the hypothesis that the result obtained was due to pilocarpine in the saliva. The record shows a marked accentuation of the pressor effect with abolition of the depression of the blood-pressure, a characteristic nicotine like action of acetylcholine in considerable doses. Addition of "pilocarpine" saliva, untreated and after hydrolysis with alkali, to the isolated small intestine in Ringer-Locke solution gave results identical with Figs. 4 and 5 obtained with "nerve" saliva.

This group of experiments on the secretion of saliva in response to pilocarpine is of interest in that it suggests the liberation of acetylcholine

as a primary response of the parasympathetic nerves to the drug. It is not a case of pilocarpine and acetylcholine having similar actions but that pilocarpine has its effects on the body by stimulating the neurones to liberate acetylcholine. This suggestion is in accord with the work of Rosin [1929], who demonstrated that while muscarine and atropine produced their characteristic effects on the frog's heart 3 weeks after double vagotomy pilocarpine was ineffective. He concluded that pilocarpine produces its effects on the heart by acting on the endings of the preganglionic fibres of the vagus.

The view expressed here is not in agreement with the findings of Anderson [1905] who concluded from his experiments on the action of pilocarpine on the denervated iris that the drug acts directly on the muscle, and not through the oculomotor nerve.

Discussion.

The results obtained in this investigation prove that a substance, resembling acetylcholine in its pharmacological actions, is present in the saliva produced in response to electrical or pilocarpine stimulation of the chorda-tympani in the cat.

It is not possible at this stage to say that the entire effects on the vascular system are due to acetylcholine, since in certain experiments there is a more marked pressor effect preceding the depression (cf. Figs. 1, 8). The possibility of the presence of some sympathomimetic substance cannot be ignored, as in these experiments no attempt was made by section of the cervical sympathetic nerve or ligation of the suprarenals, to prevent sympathetic interference. This aspect of the problem is being investigated.

The results of the experiments described here differ from those obtained by Babkin, Alley and Stavraky in that eserine did not cause a secretion of saliva. This difference may possibly be due to the different anæsthetic used in the two series of experiments. Babkin and his co-workers used a mixture of urethane and chloralose, whereas here sodium veronal was the drug used. That the anæsthetic used had an influence on the results of these experiments seemed likely, and in order to test the effect of the anæsthetic on the results of giving eserine, ether alone was used in one experiment. In this case administration of eserine was followed after 10 min. by a slow secretion of saliva lasting with diminishing rate for about 15 min. However, the clean start and finish of secretion obtained under veronal anæsthesia made it a convenient drug to use in

this work. It does not seem unjustifiable to conclude that chorda stimulation results in the liberation of acetylcholine at the nerve terminals in the gland cells with a consequent excitation of the secretory function, and that the activating substance is washed out from the gland in the secretion, so preventing continued excitement, the material produced in the blood vessels being removed by the blood stream and destroyed by enzyme action in the blood.

Summary.

1. Intravenous injection of saliva from the submaxillary gland in the cat causes marked changes in the arterial blood-pressure.

2. The active substance behaves in a manner similar to acetylcholine.

3. Eserine causes intensification of the results obtained on nerve stimulation and causes the gland to secrete on injection of saliva.

4. Saliva produced in response to pilocarpine stimulation behaves in a manner similar to saliva produced in response to nerve stimulation and the active substance appears to be acetylcholine.

I wish to express my thanks to Prof. Burns for his criticism and interest during the progress of this work and to Dr J. A. Saunders and Dr F. J. Elliott for their help in the experimental work.

I have also to acknowledge with thanks that the expenses of this work were defrayed by a grant to Prof. Burns from the Medical Research Council.

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612.352.12:612.45

THE ADRENALS AND GLUCOSE TOLERANCE.

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(*Received December* 22, 1933.)

It is clear from the work of many observers that carbohydrate metabolism is interfered with to some extent after adrenalectomy. Biedl [1913] concluded from data collected from many sources that the adrenal capsules affected in some way the formation and utilization of glycogen. More recently Cori and Cori [1928] reported that adrenalectomized rats and mice showed an almost complete disappearance of liver glycogen when fasted for 24 hours, but muscle glycogen was not much more reduced than in starved normal controls. Britton, Calvery and Geiling [1928] found an increased sensitivity to insulin in cats in which the function of adrenaline was suppressed by removing the adrenal medulla. The present investigation deals with the relationship of the cortex and medulla to the fasting blood-sugar concentration and to the glucose tolerance of rabbits.

Methods.

Two series of rabbits were prepared. In one (15) the adrenals were removed aseptically through the lumbar extra-peritoneal route by two-stage operations at intervals of not less than 16 days, and in the other (16) the medullas were destroyed by curettage in two-stage operations at similar intervals. Operative and other details have already been given by us [1933].

Glucose tolerance was determined by repeated tests at intervals of several days during the post-absorptive state before operation and after the second operation. Blood volume was calculated by the formula of Dreyer and Ray [1911], viz. blood volume in c.c. $= \frac{(\text{weight in g.})^{0.72}}{2.37}$, and glucose, 1 g./100 c.c. blood, in saline at body temperature was injected

into the marginal ear vein from a 5 c.c. syringe, which was carefully calibrated. It was convenient to dilate the ear blood vessels by xylene before the injections and before taking the samples of blood. For these purposes different ears were used.

Rabbits between 1·0 and 1·8 kg. weight were used and the standard laboratory diet was given throughout. The animals belonged mainly to the albino Himalayan variety. They were fasted in all cases for 24 hours before their glucose tolerance was tested or their blood examined. Weight records and blood records were kept throughout, *e.g.* specific gravity by the chloroform-benzene method, red-blood corpuscle counts and determination of the hæmoglobin percentage by colorimetry.

In the glucose tolerance tests, blood samples were taken before and 15, 60, 90 min. after injection and analysed in duplicate by the method of Hagedorn and Jensen [1923]. In all experiments the animals were weighed on the test day and the glucose determined accordingly. More frequent blood samples were taken in several experiments, but appeared not to be necessary.

Reid [1933] found that between 60 and 70 p.c. of rabbits of this species might survive total adrenalectomy for long periods (over 100 days) in apparently normal health, and the remainder died from acute adrenal insufficiency in from 9 to 17 days. Moreover, examination of the blood revealed in the latter group large differences from normal or from the remaining adrenalectomized rabbits, *e.g.* low fasting blood-sugar percentage as compared with the pre-operative fasting percentage and increased specific gravity. Accordingly results from rabbits which died from cortical insufficiency are dealt with separately, and these experiments may be considered in three groups.

Group I.

Those in which both adrenals were removed, and the rabbits died in a short time with signs of grave adrenal insufficiency.

Group II.

Those in which the animals survived for a long time after the same operation without any such signs.

Group III.

Those in which the medulla was destroyed but the cortex left as far as possible uninjured.

Results.

Group I.

Of fifteen rabbits subjected to two-stage total adrenalectomy, four died in the second week after the second operation. Their mean period of survival was 11 days.

The mean specific gravity of the blood of these animals before operation was 1·047 (1·044–1·049) and during the period of signs of adrenal insufficiency 1·063 (1·057–1·067). The number of red-blood corpuscles per c.mm. and the haemoglobin percentage were increased approximately 15–20 p.c.

The average loss of weight at or shortly before death was 27 p.c.

The fasting blood-sugar level was lowered after the two-stage operations. Low percentages, *e.g.* below 70 mg./100 c.c., were found repeatedly, but not in one animal which had repeatedly a fasting blood-sugar of about 150 mg./100 c.c. before the operations. It should be noted that the actual percentages, when the animals were showing signs of grave cortical insufficiency, are not shown in Table I. This shows merely the mean fasting blood-sugar level found at the tests after operation.

Table I. Group I. Blood glucose: mean of four mean values obtained by repeated tests on four rabbits: ♂ 2; ♀ 2.

	(*a*) Before operation.					
	Initial	15 min.	60 min.	90 min.	Av. weight in kg.	Av. dose of glucose in g.
mg./100 c.c.	110	283	144	106	1·23	0·724
	(*b*) After double adrenalectomy.					
mg./100 c.c.	81	181	112	76	1·08	0·685

In the intact animals of this group 76 p.c. of the injected sugar disappeared in 15 min. and 95·3 p.c. in an hour, whereas, after total adrenalectomy, 85·7 and 95·6 were the respective percentages. This indicates that the increased rate, as compared with that of the intact animals, of the removal of glucose in the 15 min. period after injection was not maintained.

Group II.

Seven of eleven rabbits in this group were examined by more than two glucose tolerance tests on each before and after operation. The remaining four were not tested so frequently, and are not included in the results summarized in Table II.

The fasting blood-sugar level of the operated animals was found to be

TABLE II. Group II. Blood glucose: mean of seven mean values obtained by repeated tests on seven rabbits: ♂ 2; ♀ 5.

	Initial	15 min.	60 min.	90 min.	Av. weight in kg.	Av. dose of glucose in g.
		(a) Before operation.				
mg./100 c.c.	109	254	117	105	1·40	0·801
Standard deviation	9·9	27·1	13·1	7·6	—	—
Standard error	3·9	11·0	5·3	3·1	—	—
		(b) After total adrenalectomy (survival).				
mg./100 c.c.	102	236	118	99	1·30	0·733
Standard deviation	8·1	17·8	18·6	5·6	—	—
Standard error	3·3	7·2	7·6	2·1	—	—

slightly lower than in the intact animals. The average decrease is held not to be significant, and is probably merely incidental to the comparative absence of secretion of adrenaline in an easily excitable animal like the rabbit.

Of the injected sugar 82 and 99 p.c. disappeared from the circulation in 15 and 60 min. respectively. After operation the respective percentages were 81·7 and 98.

The blood was apparently normal, and weight records of the animals showed no change or a slight fall. Their general health was good.

Group III.

In this group eight out of sixteen rabbits were examined by more than two tests on each before and after the operations. Results from the remaining animals, although confirmatory of Table III, are not included.

TABLE III. Group III. Blood glucose: mean of eight values obtained by repeated tests on eight rabbits: ♂ 3; ♀ 5.

	Initial	15 min.	60 min.	90 min.	Av. weight in kg.	Av. dose of glucose in g.
		(a) Before operation.				
mg./100 c.c.	110	249	124	105	1·21	0·699
Standard deviation	4·4	16·5	17·3	3·5	—	—
Standard error	1·6	6·1	6·4	1·3	—	—
		(b) After operation (destruction of the medulla).				
mg./100 c.c.	104	232	126	101	1·13	0·666
Standard deviation	6·0	14·0	13·0	4·7	—	—
Standard error	2·2	5·2	4·8	1·7	—	—

The average fasting blood-sugar level was slightly lower after the operations, but the decrease is not considered significant.

In the course of 15 and 60 min. respectively 80 and 98 p.c. of the injected sugar had disappeared from the blood of the intact animals. After operation, these percentages were not substantially altered, viz. 81 and 97.

Blood and weight records of this group revealed no significant change during the period of observation.

Discussion.

Group I.

It is clear that the fasting blood-sugar level was below normal in the animals after the two-stage adrenalectomy. This holds in spite of concentration of the blood which of itself would tend to mask the decline in the blood sugar. As the blood of the rabbits in Group II was normal apparently during our periods of observation, these changes in the blood must be related to cortical insufficiency.

The mean specific gravity of the blood of this group was 1·047 before the operations and 1·063 during the period of grave cortical insufficiency. In order that the specific gravity of the blood should rise from 1·047 to 1·063 without an actual concentration of the plasma, an increase in the volume ratio of red-blood corpuscles to plasma from 1 : 1 to 4 : 1 would be necessary. The average increase in the number of red-blood corpuscles per c.mm., however, was about 20 p.c., and this would bring the ratio of corpuscles to plasma to about 3 : 2. Hence, presumably, an actual increase in the specific gravity of the blood occurred due to (1) a diminished water content, or (2) an increased solid content. The fluid intake of the animals with signs of adrenal insufficiency is undoubtedly diminished owing to loss of appetite. This would explain largely or wholly the finding of an increased concentration of the blood. This point was not decided conclusively, but it was suggestive to find that the amount of blood which was obtained in two rabbits dying from adrenal insufficiency was much less than from two controls of similar weight.

It is clear that the tissues of the operated rabbits of this group retained their ability to remove sugar from the circulating blood, and that this ability was somewhat greater than normal for some minutes after the glucose injection. There is no evidence, however, that adrenalectomy accelerated or retarded significantly the arrival of the blood sugar at the fasting level again after injection as compared with what held good for

intact rabbits. Moreover, a slight degree of hypoglycæmia of similar order was evident at 90 min. in both intact and operated animals.

The average dose of glucose given after the two-stage operations was 0·685 g. For an average post-operative weight of 1·08 kg. the dose should have been 0·654 g. In several of the tests the amount of glucose given was the same after as before the operations, in spite of some loss of weight suffered by the animals. This procedure meets a point which might be made that, if the loss of weight were largely due to loss of fat from the fat depots, the more rapid disappearance of glucose in the operated animals is due largely or wholly to the smaller dose injected.

Glucose is a diffusible substance and the gradients of diffusion of the glucose molecule in and out of the blood would depend on the ratio of plasma sugar to tissue sugar. This is clear from Cori's [1931] experiments in intravenous glucose injections on rats with high blood-sugar values. MacGrath [1927], working on the carbohydrate balance of fasted mice, found blood sugar and tissue sugar to be 123 and 54 mg./100 g. body weight, and, 30 min. after insulin, 66 and 26 mg. respectively. The lowered blood-sugar values found by us in rabbits with acute signs of adrenal insufficiency are taken as indicating depletion of tissue sugar and the initial more rapid disappearance of injected glucose from their blood as confirmatory of this conclusion.

Further, we have shown [1933] that anæsthetics, such as ether, can increase the blood sugar in adrenalectomized rabbits with cortical insufficiency. This hyperglycæmia is presumably the result of glycogenolytic processes. Since glycogenolysis can occur in adrenal insufficiency and the tissues are capable of removing injected sugar rapidly from the blood, it seems a reasonable conclusion that the adrenal cortex is concerned with the retention of carbohydrate in the organism. Moreover, the results of Kuriyama [1918] and of Cori and Cori [1928] indicate that the adrenal cortex is partly responsible in some way for the formation and retention of glycogen in the liver rather than in the muscles. In spite of this finding and in the absence of clear evidence of the influence of cortical extracts on carbohydrate metabolism, the connection of the adrenal cortex with carbohydrate metabolism may be simply an indirect one in that the cortex supplies some component necessary for the vital processes of the organism.

Adrenalectomy is not without effect upon the circulatory organs. Elliott [1914] found an almost complete paralysis of the vaso-motor mechanism and the cardio-accelerator nerves. We observed [1933] that the heart of rabbits, with signs of cortical insufficiency, whether anæs-

thetized or not, is feeble and slower than normal. This would result in a decreased cardiac minute output. It is a moot point whether diffusion of injected glucose from the blood of those animals with slow circulation rates would proceed more quickly than the diffusion of glucose injected in corresponding dose from the blood of animals with normal circulation rates.

In adrenalectomized animals secretion of adrenaline must be suppressed except in so far as the scattered chromaphil tissue is concerned. Since a disturbance of the glucose tolerance test was not effected by removing the adrenal medulla (Table III), the suppression of medullary function alone could not account for the results obtained from the glucose tolerance test in our animals which died later from cortical insufficiency.

A further consideration arises in connection with the extraordinary sensitivity to insulin noted by Britton, Calvery and Geiling [1928] after destruction of the medulla in cats. These animals had normal amounts of sugar in their fasting blood and of liver glycogen. It is unlikely that this could account for the low figure recorded at 15 min. after injection of glucose in our adrenalectomized animals. Further points against this being a predominant factor in our results are that, in all our groups of operated animals, (1) there is little or no evidence of a significantly earlier return of the blood sugar to the normal fasting level after glucose injections, and (2) a more decided hypoglycæmia would have been expected than in the intact animals. Such, however, was not the case.

It is unlikely that the increased rate of disappearance of glucose from the blood in the first 15 min. in Group I could be due to increased loss in the urine as compared with normal. This point was not tested repeatedly and, in any case, would have significance only if the loss of sugar was decidedly higher in the adrenalectomized animal during the first 15 min. after injection. In one animal, after anæsthetizing the skin of the suprapubic region by cocaine, a fine trocar with cannula was inserted into the bladder, tied on and clipped off. The bladder could then be emptied easily by aspiration and washed out with sterile saline immediately before the glucose injection and again at 15 min. after the injection or subsequently, and the urine tested quantitatively for sugar. The difference of a few milligrammes in the amount of sugar excreted in the urine of the intact and the same animal adrenalectomized was held to be of no import as regards the significance of the difference in the increases in blood sugar at 15 min.

Groups II and III.

It is clear that the glucose tolerance of rabbits which come into these groups is not disturbed significantly by removal of the entire adrenal

tissue or by destruction of the medulla with preservation of the cortex as far as possible. The decrease in the fasting blood sugar is held to be slight, and probably is merely incidental to the comparative absence of secretion of adrenaline in an excitable animal.

As regards the decreased values of the mean found at 15 min. after injection in the operated animals, four factors would have to be considered: (1) smaller average amount of glucose injected owing to the slight decrease in the mean weight; (2) decreased cortical tissue leading to a mild form of cortical insufficiency; (3) increased sensitivity to insulin; (4) suppression of adrenaline secretion. In regard to (1), the dose of glucose was calculated as recommended by Orr-Ewing [1931] who found that consistent results in glucose tolerance tests in rabbits were obtained by giving glucose in amount depending on the calculated blood volume [Dreyer and Ray, 1911] rather than body weight. Decreased cortical tissue might be a factor, but the rabbits were apparently in normal health and neither body weight nor examinations of the blood revealed signs of grave adrenal insufficiency. Increased sensitivity to the amount of insulin secreted during the post-injection hyperglycæmia is unlikely to account for the difference at 15 min., as significant differences in the means at 60 and 90 min. were not obtained. The decreased means at 15 min. may, therefore, be largely due to the absence of the adrenalinæmia which would accompany ordinarily the injection in intact animals. In any case, the standard error of the means at 15 min. is considerable.

Summary.

1. Fasting blood sugar has been determined and the glucose tolerance of normal rabbits tested by intravenous injection and compared with that of rabbits (*a*) after total adrenalectomy resulting in insufficiency; (*b*) after this operation without signs of cortical insufficiency; (*c*) after destruction of the medulla alone.

2. The fasting blood sugar of the rabbits with adrenal cortical insufficiency was found to be lower than normal, and slightly lower in the remaining groups of operated rabbits. It is pointed out that the slight decrease in the latter may be merely incidental to the suppression of the function of the medulla.

3. Injected glucose disappeared rapidly from the blood of both normal and operated rabbits.

4. It appeared that, in the course of 15 min. after injection, glucose disappeared more rapidly from the blood of animals with signs of cortical

insufficiency, as judged by the decrease in fasting blood sugar and in body weight and by the increased specific gravity of the blood. This increased rate of disappearance was not maintained.

5. The glucose tolerance of rabbits surviving adrenalectomy for long and of those in which the medulla alone was removed did not differ significantly from that of the intact animals.

6. It is concluded that the tissue sugar of rabbits with signs of cortical insufficiency is low, but that the tissues have retained their ability to remove injected glucose from the circulating blood.

Part of the expenses of the above investigation was met by a grant from the Indian Research Fund Association to whom our thanks are due.

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612.617.7:577.174.5

AN ADRENALINE-LIKE ACTION IN EXTRACTS FROM THE PROSTATIC AND RELATED GLANDS.

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(*Received January* 17, 1934.)

In continuation of investigations as to a depressor substance in certain tissue extracts, the presence of which has been previously announced in this *Journal* [Euler and Gaddum, 1931], extracts from the prostatic and other glands of the male genital apparatus were also tested. Extracts from the vesicular glands of sheep and steer, like prostate extracts from man, dog, rabbit, and guinea-pig, but unlike most other tissue extracts, not infrequently showed a pressor action on the rabbit's blood-pressure when tests for the substance mentioned above were made. This pressor action was investigated further, evidence being obtained for the assumption that this action was due to a substance in its actions closely resembling adrenaline.

Earlier investigators have observed remarkable actions of extracts of the prostate glands from different animals. Thus Jappelli and Scafa [1906] found that such extracts from dogs produced a pressor action on the dog's blood-pressure and stimulation of respiration, after intravenous injection. Rabbits reacted with a lowering of the blood-pressure. They found, too, that these extracts, made up with saline from the fresh glands, were highly toxic to the animals. A pressor action was obtained by Thaon [1907] after intravenous injection of prostatic extracts from steer and dog on the rabbit's blood-pressure, in most cases followed by a strong depressor action. These injections not infrequently ended in the death of the test animal. (The term "prostatic gland" is probably used for the vesicular gland of the steer [cf. Disselhorst, 1904].) The hypertensive action was destroyed when the extracts were heated for one hour at 54° C. Other effects described after intravenous injections of prostatic and similar extracts are contraction of the urinary bladder and, in most cases, an inhibitory action on the isolated intestine of certain animals

[Dubois and Boulet, 1911, 1919]. A pharmaco-dynamical analysis of the actions observed does not seem to have been performed, and it is hardly possible, from the descriptions of the experimental results, to draw any conclusions with regard to the exact nature of the active principles in the extracts. Pharmacologically active extracts from the prostatic gland have also been prepared by Macht and Matsumoto [1920]. The lack of specific actions leads Macht and his co-worker to doubt any special function of the prostate gland as an endocrine organ with regard to the contractions and tonicity of genito-urinary organs.

Experimental.

The extracts used in this investigation were all made up of 1 part of finely minced fresh organ and 2 parts of alcohol, with the addition of 2 c.c. of $2N$ H_2SO_4 for each 100 g. of tissue. The alcohol was then evaporated under low pressure, the temperature not exceeding 25° C. The extracts thus obtained usually had a milky appearance, due to the presence of lipoids which could easily be removed by ether extraction without altering the effect of the extracts. The acidity of the extracts, after reducing the volume to an amount corresponding to 0·5–4 g. of tissue per c.c., was usually slightly acid (*p*H about 4–5). If for some reason the reaction was more acid than would correspond to *p*H 3, the reaction was adjusted by means of NaOH to *p*H 4–5. This mode of preparation corresponds in the main to that used by Euler and Gaddum. All extracts were kept in an ice chest at a temperature not exceeding +5° C. in the cases where they could not be tested immediately.

The test animals were in most cases male rabbits, and in some experiments cats, which were narcotized with ethyl urethane, 1·5 g. per kg., or with ether. Effects of choline and allied substances were prevented by atropine in a dose of 2 mg. per kg. subcutaneously. All injections of extracts were made intravenously through a cannula inserted in the jugular vein of one side. The blood-pressure was recorded by means of a mercury manometer in the carotid artery. Attention was paid to the mode of injection, which was kept as uniform as possible.

Prostatic extracts were made from organs from man and the following animals: dog, rabbit, pig, and guinea-pig. Extracts were further made from the vesicular glands of steer, sheep and pig, from the ampulla ductus deferentis of dog, steer and sheep, and from the Cowperian glands of sheep and pig. In referring to the different glands and organs belonging to the male genital apparatus the nomenclature of Disselhorst has been followed. The extracts from the organs of rabbit, guinea-pig and

in some cases dog were prepared immediately after the death of the animal, and those from the other animals within ½–1 hour.

Pressor actions and also strong inhibitory actions on the isolated rabbit's gut were obtained with extracts from the prostatic gland of man (obtained at autopsy, and, in some cases, after surgical enucleation), dog, rabbit and guinea-pig and with extracts from the vesicular glands of steer and sheep, and also with extracts from the ampulla ductus deferentis of dog, steer and sheep. No distinct pressor effect was observed with extracts from the vesicular, prostatic or the Cowperian gland of the pig (one experiment) or with the Cowperian glands of the sheep. The fact that a pressor effect could not always be clearly detected may be due, in some cases at least, to a simultaneous action of depressor substances, which have been found to be present in most of these extracts. Different degrees of sensitivity are probably the reason for the different reactions of the test animals in some cases; thus one animal may show a much greater pressor effect than another with the same extract. The difference may be especially evident after the administration of atropine.

Some experiments with extracts from the vesicular glands of a young steer will be given in greater detail below. The actions described have been repeatedly found with all types of extracts showing a pressor action on the rabbit's blood-pressure. The vesicular gland of the steer was found to give extracts with a rather marked action. The only organs giving pressor reactions of the same order were the vesicular glands and ampullæ ductus deferentis from sheep.

Action of extracts of the vesicular gland of the steer.

The pressor effect of an extract made of the vesicular gland of the steer, with a relatively potent action, is shown in Fig. 1 at A. At B, *d*, the effect is shown with a higher dose of the same extract on another test animal. In both cases the animal was atropinized. The pressor actions differ somewhat from each other, which may occur, as indicated above. The actions differ also from that which will follow an injection of adrenaline (B, *a*). If, however, adrenaline was mixed with a depressor substance, the effect observed was more similar to that caused by the extract (cf. Fig. 1 B, *c* and *d*). The depressor substance used in this case, the action of which is shown at B, *b* in Fig. 1, was obtained from ox brain and possessed the characteristic qualities of the substance described by Euler and Gaddum. The extract was purified by extraction with alkaline absolute alcohol and afterwards further purified by electrodialysis, this treatment also showing the basic character of this substance.

In order that the pressor action observed might be analysed the animal was rendered more sensitive to adrenaline by means of cocaine

Fig. 1. Blood-pressure of rabbit, urethane, atropine.

A. 0·7 c.c. extract of glandula vesicularis of steer (147).

B. *a*, 5 γ adrenaline. *b*, 0·25 c.c. depressor extract from ox brain, purified (137). *c*, 0·25 c.c. 137 + 5 γ adrenaline. *d*, 1 c.c. extract of glandula vesicularis of steer (147). *e*, 1 c.c. 147 treated with alkali and O_2. *x*, 5 mg. cocaine hydrochloride intravenous. *f*, 1 c.c. 147. *g*, 0·25 137 + 5 γ adrenaline.

Between B and C 3 mg. ergotamine tartrate intravenous.

C. *a*, 0·25 c.c. 137 + 5 γ adrenaline. *b*, 1 c.c. 147.

Fig. 2.

A. Isolated rabbit's jejunum in Tyrode, from the same animal as in Fig. 1. *a*, 0·25 c.c. 137 + 5 γ adrenaline. *b*, 1 c.c. 147 (extract of glandula vesicularis of steer). *c*, 0·25 c.c. depressor extract (137).

B. Isolated rabbit's jejunum in Tyrode, atropine. *a*, 0·6 c.c. extract of glandula vesicularis of sheep (144 *a*). *b*, same extract treated with alkali and O_2. *c*, 0·6 c.c. 144 *a*. The extract evaporated and extracted with alkaline absolute alcohol.

in conformity to the observations first described by Fröhlich and Loewi [1910]. After intravenous administration of 5 mg. of cocaine hydrochloride, the same amount of extract showed an almost pure

pressor action (Fig. 1 B, *f*) which did not differ much from the action of the mixture made of 5 γ adrenaline and 0·25 c.c. of the depressor extract shown at *g*. From this experiment it seems that the initial action at B, *d*, may be composed of a depressor action and a pressor action, the latter being similar to that caused by adrenaline. The assumption that the pressor action at B, *f*, is due to adrenaline was strongly supported by the following experiment. Thirty minutes after the injection of cocaine, a period sufficient for the major part of the cocaine effect to disappear, the animal received 3 mg. of ergotamine tartrate intravenously in three doses at 2 min. intervals, in order to abolish or invert the adrenaline effect [Dale and Spiro, 1922]. An injection of the extract of the vesicular gland about 5 min. after the last ergotamine injection was now followed by a pure depressor action. Much the same effect was produced by an injection of the mixture of adrenaline and depressor substance in the same quantities as above (Fig. 1 C, *a* and *b*). In order to obtain further information as to the nature of the observed effects, the comparison between the two solutions as under Fig. 1 B, *c*–*g*, was extended also to the isolated rabbit's intestine. Part of the jejunum of the animal used for the blood-pressure experiment above was immersed in Tyrode's solution. As shown by Fig. 2, under A, the effect of 5 γ adrenaline mixed with 0·25 c.c. depressor extract as well as 1 c.c. of the extract give only a weak inhibition as compared with the effect ordinarily obtained (volume of bath 25 c.c.), showing that the intestine is still under the influence of ergotamine. A comparison of the effect of the vesicular gland extract (Fig. 2 A, *b*), the adrenaline mixture (A, *a*), and the depressor extract alone (A, *c*) shows further, that no great difference appears in this test, as on the blood-pressure.

From these experiments it may be regarded as probable that the pressor action and the inhibitory action on the isolated intestine are caused by a substance closely related to adrenaline or by adrenaline itself. The amount of the pressor substance present in the organ may be roughly estimated in terms of adrenaline from the data communicated. The extract used in Fig. 1 was made of 0·75 g. of vesicular gland per c.c., and, since 5 γ adrenaline mixed up with depressor substance gave an action not very different from that of 1 c.c. of the extract, it will follow that the organ contains about 0·005 mg. adrenaline or slightly more per gram of tissue approximately, if no losses during the preparation have occurred. Similar figures were obtained also with extracts from the ampulla ductus deferentis of sheep. Extracts from the prostate gland of man, dog and rabbit have given lower figures, about 1–2 γ per g. of tissue.

As to the nature of the depressor action of the extracts, it has become evident that this is primarily due to adenylic compounds but also in some cases to the active substance of Euler and Gaddum. This substance was found, not only to depress the rabbit's blood-pressure in the presence of atropine, but also to stimulate, under the same conditions, the isolated gut of the rabbit. In order to discriminate between the depressor effects of choline and choline compounds and of adenylic compounds, whose pharmacological actions were discovered by Drury and Szent-Györgyi [1929] on the one hand and the substance referred to above on the other hand some of the extracts were evaporated to dryness and extracted with alkaline absolute alcohol. This treatment will bring the substance of Euler and Gaddum in the alcoholic solution, leaving the adenylic compounds behind for the most part (Fig. 4 A, *a*, *b*), as shown by previous experiments. The alcoholic extracts, after evaporation of the alcohol and redissolving in saline in many cases showed the depressor action and stimulating effect on the isolated gut after atropine which is characteristic of the substance in question. If the amount of this substance was small as compared with the "adrenaline" content of the extracts, the latter was destroyed by treatment with alkali and O_2, which seems to act more readily on the pressor principle than on the depressor (Figs. 2 B, 3). Choline effects were excluded by means of atropine. The presence of this depressor substance in considerable amounts in extracts from certain male genital accessory glands, and also in native prostate secretion will be the subject of a special note.

Fig. 3. Rabbit's blood-pressure, urethane, atropine.
a, 0·6 c.c. extract of glandula vesicularis of sheep, re-extracted with alkaline absolute alcohol (144 *a*), treated with alkali and O_2. *b*, 0·6 c.c. 144*a* untreated. *c*, same as *a*.

In addition to the pharmacological tests indicated above for adrenaline in extracts from male accessory genital glands some evidence of chemical and histo-chemical nature has also been given. Thus the pressor effect, as well as the inhibitory action on the isolated rabbit's gut, is diminished or completely eliminated if the extracts are treated with iodine at a slightly acid reaction (*p*H about 6) or by bubbling through the extract for some minutes with oxygen in an alkaline solution (*p*H

about 11), which treatment also diminishes or eliminates the action of adrenaline in these respects (Figs. 1 B, *c*, 2 B, *b*, 3, 4 B–E). The addition of iodine has not been found to produce definite changes in the action of the depressor substance of Euler and Gaddum. If the excess of

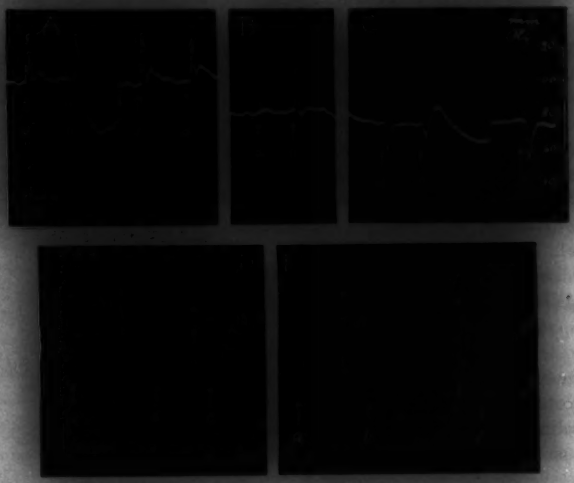

Fig. 4. A–C, rabbit's blood-pressure, urethane, atropine. D–E, isolated rabbit's jejunum.

A. *a*, 0·5 c.c. extract of glandula vesicularis of sheep, re-extracted with alkaline absolute alcohol (53 *a*). *b*, equivalent amount of extract before re-extraction. *c*, 1·6 γ adrenaline. *d*, same as *a*.

B. *a*, 1·6 γ adrenaline inactivated with iodine at *p*H 6. *b*, 1·6 γ adrenaline.

C. *a*, 0·5 c.c. extract of ampulla ductus deferentis (55 *a*) of sheep, re-extracted with alkaline absolute alcohol, treated with iodine as above. *b*, same as *a* before treatment with iodine. *c*, same as *a*.

D. *a*, 0·3 c.c. 55 *a* treated with iodine. *b*, 0·3 c.c. 55 *a*, untreated. *c*, same as *a*.

E. *a*, 0·3 c.c. extract of glandula vesicularis of sheep, re-extracted (54 *a*). *b*, 4 γ adrenaline. *c*, 0·3 54 *a* treated with iodine.

iodine is removed by means of thiosulphate, no significant actions on the rabbit's blood-pressure or on the isolated gut are observed with the amounts of iodine used. In some cases the iodine was to a considerable part reduced, either immediately or after a while; in all cases iodine therefore was added until a small excess was reached. The treatment of

the extracts with oxygen-bubbling in an alkaline solution caused sometimes losses of the amount of the depressor substance.

The histological finding by the author of groups of chromaffine cells in the muscular wall of the ampulla ductus deferentis of the dog and also in the prostate gland of the cat (cf. Fig. 5) represents a strong further support for the assumption that these organs are adrenaline-producing. The preparations were obtained by treating the tissue as

Fig. 5. Photograph of prostate gland of cat showing chromaffine cells at *B* and glandular cells at *A*. × 150.

soon as possible after death with 2·5 p.c. potassium bichromate solution, which was replaced after 3 days by a bichromate-formol solution for one day [Borberg, 1912]. The preparations were stained with toluidine blue, causing the chromaffine cells to appear in a distinct light green colour. The brown colour of the cells could be directly seen on adjacent sections in otherwise unstained preparations.

No reasons for the possible existence of an adrenaline-like substance which is not adrenaline itself have been observed. On the contrary, the identification of the pressor substance received further confirmation, as

it could be shown that highly active extracts gave the Vulpian reaction with iodine. In a few cases the amount of the adrenaline-like substance was estimated colorimetrically by the method of Euler [1933] and also biologically. In the case of an extract from the vesicular glands of the steer the amount determined colorimetrically as adrenaline was 0·71 mg. in 260 g. of organ and the amount estimated biologically was between 0·65 and 0·87 mg. In view of the difficulties of obtaining an exact biological estimate with these extracts, which also show other effects, the figures correspond fairly well. The colorimetrical determinations were made with extracts prepared after precipitation of the proteins with trichloracetic acid.

No attempts have been made to isolate the active pressor substance or to isolate adrenaline from these organs, owing to lack of sufficient amounts of organ material.

Discussion.

The presence of chromaffine cells has been described—apart from the suprarenals—also in the sympathetic by-organs, such as the ganglion aorticum (organ of Zuckerkandl), in the carotid gland and in the coccygeal gland. Small groups of chromaffine cells have also been observed in sympathetic ganglia in other parts of the body, for instance in the thoracic ganglia. The finding of groups of chromaffine cells in the muscular wall of any organs does not seem to have been described before. The fact that the prostatic gland and also the ampulla ductus deferentis is surrounded by sympathetic ganglia is, however, well known. The presence of such ganglia in the neighbourhood may open the way for the criticism that the effects observed are mainly due to the adrenaline present in the adjacent sympathetic tissues, which may not have been separated carefully enough before the extraction. Apart from the fact that all surrounding tissues were carefully eliminated, the finding of groups of chromaffine cells within the muscular wall of an organ giving potent pressor extracts will reduce the strength of such criticism. Of other organs around the prostate gland neither the urinary bladder nor the Cowperian glands have shown pressor effects or such actions on the rabbit's isolated gut as could possibly be explained as due to adrenaline. These facts definitely speak against the assumption that surrounding sympathetic tissue, which will be found around the urinary bladder as well, is the cause of the adrenaline-like actions. The latter seems, from the experiments with extracts from other organs in this region, to be of specific nature, as they only appear in extracts from the prostatic and

the vesicular gland, and from the ampulla ductus deferentis. Extracts from the testis of steer and sheep were not found to contain adrenaline in such amounts as could be demonstrated in the way described here. Of other extracts only ovaries from the cow have shown pressor effects.

Other experiments indicating the presence of adrenaline in organs and tissues may be mentioned here. Thus the adrenaline-like action demonstrated by Loewi [1922] in his classical experiments on the frog's heart, the "sympathin" of Cannon and Bacq [1931], and the actions reported by Finkleman [1930] may be caused by adrenaline present in the respective organs and tissues. Chromaffine cells do not seem to have been observed in these cases, which may be due to the absence of considerable groups of these cells.

The presence of such amounts of an adrenaline-like substance as has been found in the extracts referred to in this paper seems to indicate a special function of these organs as adrenaline-producing. Opinions have differed as to the question whether the prostate gland has a function as an endocrine organ. Most investigators and reviewers [Fischer, 1932; Romeis, 1926] have taken the standpoint that sufficient evidence has not been produced to allow of such a theory. By the demonstration of a substance probably identical with adrenaline in comparatively large amounts in this organ, the assumption of an endocrine function is rendered more likely. The possible function of some of the male accessory genital glands as endocrine organs might then be regarded in connection with the physiology of these organs, and especially in view of the fact that adrenaline, like stimulation of the sympathetic nerves in this region, will cause contraction of the plain muscle in general of the genital apparatus [lit. see Trendelenburg, 1924; Weil, 1926]. The influence of times of rut, sexual activity, age, etc., upon the content and secretion of the active substance regarded as adrenaline in the genital accessory glands may contribute to the understanding of these observations and some physiological phenomena with regard to the sexual functions.

Summary.

In watery alcoholic extracts from the prostate glands of man, dog, rabbit, guinea-pig, from the vesicular glands of steer and sheep, and from the ampulla ductus deferentis of dog, steer and sheep, pressor action and inhibitory action on the isolated rabbit's gut have been observed.

Inactivation experiments, the presence of the Vulpian iodine reaction in the extracts, and the finding of chromaffine cells within the muscular wall of the ampulla ductus deferentis and the prostate gland

as well as characteristic changes in action after cocaine and ergotamine, indicate that the actions observed are due to adrenaline.

The amount of the active substance corresponds to 0·001 to about 0·005 mg. of adrenaline per gram of fresh organ.

The bearing of these observations upon the physiological functions of the male accessory genital apparatus is briefly suggested.

The expenses of this investigation have been defrayed by a grant from the Foundation "Thérèse och Johan Andersons Minne."

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612.817.1

NEURO-MUSCULAR ISOCHRONISM AND CHRONOLOGICAL THEORY OF CURARIZATION.

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(*Received January* 28, 1934.)

In a recent paper Rushton [1933], thinking he had refuted in previous papers the conception of neuro-muscular isochronism, attacked the theory of curarization by heterochronism. This is logical. The two conceptions are closely bound together—they have been developed in conjunction with one another, and I have arrived simultaneously at both conclusions by a consideration of chronaxie in various forms of curarization. Actually, it appears difficult to separate these two conceptions in the mind.

Strictly speaking, it would be possible to admit isochronism as a perfectly normal state of affairs, and also curarization depending on some mechanism related to the old classical theory, *i.e.* poisoning of nerve endings, without any change of chronaxie or at least any significant change. But then, isochronism, which in my view constitutes a most remarkable functional adaptation created and maintained by the interaction of two elements, would merely be an odd coincidence. Otherwise, if the muscle had normally a very different chronaxie to that of its motor nerve, it is quite clear that isochronism would not be a necessary condition for the propagation of impulses from one tissue to another, and one could not explain by heterochronism the abolition of this conduction.

The discussion must therefore embrace these two arguments, inasmuch as Rushton has started from one and the same experimental ground for the denial of isochronism on the one hand and the chronological curarization theory on the other, *i.e.* the peculiar appearance of muscular excitability under particular conditions given by electric stimulation through a large electrolytic bath. The strength-duration curve in that case proves to be the so-called α curve, which admits, for swift muscles, much more elongated excitation spaces than for the corresponding nerves, and does not vary when the muscle is poisoned with curare. Both facts are unquestionable; I admitted and experimentally confirmed the first as soon as Rushton reopened the matter, in 1930,

and the second fact was emphasized by myself a few months later, in 1931. They are interesting, and worthy of study, but are they of such fundamental importance as to reverse the bulk of knowledge on excitability, founded upon stimulation in air? A year or so before the start of the present controversy, Craib [1928] showed that the action current of a muscle, in the same condition giving birth to the α curve, *i.e.* immersion in Ringer, is distorted in such a manner as to become almost unrecognizable; neither Craib nor anyone else, as far as I know, has concluded that, on these grounds, one ought to reject the classical curve. It seems more advisable to try to find out whether such results are not due to an abnormal process under special conditions. As to the α curve, I have succeeded, I think, in demonstrating that it is the effect of a purely physical process, to which I have given the name of "retrograde polarization," and not at all a chronological picture of muscular excitability.

I have to thank this *Journal* for having opened its pages to four papers on the subject [Lapicque, 1931*a*, 1931*b*, 1932, 1933*a*]. I confess that my demonstration, although on the right track, was rather laborious in the beginning; but it acquired progressively more and more precision and was brought more and more to bear upon direct physiological evidence.

So I can leave aside here, for the sake of brevity, the discussion of Rushton's criticism of my physical reasoning, I am planning to resume it elsewhere. Nevertheless, I must say at once that this criticism has no bearing upon my theory. Rushton simply did not understand what I meant by "retrograde polarization." I should perhaps call it "retrograde displacement of polarization." But Rushton is quite mistaken when he thinks that I "have tacitly rejected the classical connection between polarization and excitation." This may be due to his more or less false ideas of classical definitions, *e.g.* a confusion of electrotonus in nerve with diffusion according to Nernst's theory. But whatever reasoning is adopted the aim was to elucidate the discrepancy between the chronological curves given by the large electrodes on the one hand, and the small ones on the other: I came to the conclusion that a systematic error was introduced by large electrodes, and Rushton to the opposite. Now, here are the results of physiological experiments.

To express them objectively, we must lay down a definition. No matter under what conditions stimulation is applied, a strength-duration curve of roughly the same form is obtained; for long current durations, the liminal intensity remains constant (rheobase); below a given duration, the intensity rises more and more as the duration diminishes. Consequently a duration corresponding to the threshold for an intensity double that of the rheobase can always be found. This duration is a chronaxie, if the strength-duration

curve is entirely and exclusively determined by the physiological properties of the living substance under experiment; but if any physical process, taking place between our electrical circuit and the physiological reaction, introduces any deformation in the curve, the liminal duration for the double of the rheobase evidently no longer represents the exact chronological character of the living substance; this duration is a false chronaxie, or rather it is not a chronaxie at all. How can we refer to it?

Rushton [1932], although considering it rather laughable "to imagine that certain values of chronaxie are truer than others," has agreed to restrict the word chronaxie to such cases which I intend to decree as such, "since I was responsible for the name in the first place"; and in order to free himself from my intolerance, he proposed to adopt, as a general term, excitation time. Such a term has no precise meaning; with any curve one finds a different excitation time for every different duration of current. We should add, to explain Rushton's intention, "Excitation Time for a strength equal to 2 Rheobases." Let us adopt the abbreviation $ET2R$, an expression with which we can compare roughly in terms of time various curves, even heterogeneous ones.

A. On one and the same muscle, actually the aneural part of the frog's sartorius, *i.e.* pure muscular substance, $ET2R$ varies systematically with the size of the active electrode. From 1 mm. upwards $ET2R$ increases almost linearly, up to a rather vague limit with quite different electrodes (a whole α curve), about 10–20σ with Rushton's block. Below 1 mm., $ET2R$ does not tend towards 0, with the size of the electrode, as Rushton's theory would require it, but the curve bends to a constant level, practically reached at an electrode size of half a millimetre. That is to say, there is a range of electrode sizes, still very great in comparison to the muscular elements, in which $ET2R$ is independent of the size [Lapicque, 1932]. Nor does it depend on the nature of the electrode, since one gets the same figure with a metallic wire in air, and with a capillary electrode, either in air or under a Ringer bath.

On a normal frog sartorius the figure is about 0·3σ.

B. On a series of muscles of various rapidities, as judged from the contraction, $ET2R$ given by diffuse electrodes (α curve) does not vary systematically from one muscle to another; under Rushton's block its value is constantly 10–20σ for the swift sartorius, and it is the same for the *Holothuria* muscle, which is 40 times slower. With electrodes of limited size tending towards zero, $ET2R$ decreases only very little on slow muscles, more on intermediate muscles, the curve in each case tending to a minimum, which is higher the slower the muscle. So that the constant value reached with the range of small electrodes classes the muscles in the same order as the duration of their twitch [Lapicque, 1933 *a*].

I must add a confirmative fact which I had overlooked; as early as 1924, that is to say immediately after Davis [1923] and Watts [1924] had raised the question of electrode size, Adrian [1925] published a result quite in accordance with the preceding statement.

On the frog's ventricular muscle, notoriously endowed with a relatively slow excitability, electrodes of various sizes of the order of 1 mm. (cotton-wool pads) down to microscopic sizes (Pratt's pore electrodes or metallic pointed electrodes) give about the same value, 5σ, although quite diffuse electrodes give much larger *ET2R*. Adrian inferred, as we do now, that the constant value given within the range of small electrodes "is more truly representative of the ventricular muscle."

Quite recently, we have resumed experiments on the heart with a series of capillary electrodes similar to those used in our experiments on various muscles quoted above. The results are various *ET2R*, from 4 to 20σ, depending on the stimulated tissue, whether myocard or Gaskell bundle, either on frog or turtle, but for a given tissue no alteration takes place when the electrode diameter is changed from 0·2 to nearly 3 mm. [L. and M. Lapicque, 1933*b*]. All this is perfectly consistent.

C. Undoubtedly a muscle is accelerated in all its processes when it is heated, and is slowed down when it is cooled. The curve of the muscle twitch undergoes in this sense well-known modifications. Nearly 40 years ago, Gotch and Macdonald [1896] showed that a cooled motor nerve becomes less sensitive to short stimuli and more sensitive to long. This is quite clearly expressed, in the modern conception of excitability, by an increase of the chronaxie, and a lowering of the rheobase [Lapicque, 1926, p. 231]; the same changes, even more marked, are noticeable on the aneural part of the sartorius under a small electrode. But stimulation by a large fluid electrode reveals no modification on the same muscle. The α curve is not altered or, if it is, in a reverse direction; *ET2R* diminishes slightly when the temperature is lowered, and increases when the temperature rises [Benoit, 1933].

Lucas and Mines [1907], on the sartorius with Lucas electrodes, found unquestionably an increase in *ET2R* for lower temperature; we are dealing here with α curves, because experiments are made under the same conditions under which Lucas discovered this α curve. But it must be remembered that Rushton's α is much longer than the Lucas α, not only "somewhat longer" as Rushton has observed in his first paper [Rushton, 1930, p. 321]. Rushton adds that the length of fibres exposed to the stimulus accounts for this difference; this hypothesis is correct, though wrongly based upon a comparison with Cardot and Laugier's experiments on electrode distance on the nerve. I have estimated, from my own experiments, the relation of the *ET2R* to the probable length of sartorius exposed to retrograde polarization in Lucas's experiments, and found to all appearances an agreement with the empirical rule, established upon other experimental grounds, 1σ more for each millimetre. Matton [1932], in my laboratory, undertook direct researches on this point; he states that *ET2R* varies systematically, though not quite regularly, with the length of muscle (pelvic end) left inside the cathode, *e.g.* from $1{\cdot}9\sigma$ for 2 mm. to $5{\cdot}2\sigma$ for 5·8 mm. In his experiments at ordinary temperature, Benoit found for *ET2R* values between 15 and 20σ, in full agreement with Rushton's figures. Consequently, Lucas's α is intermediate between Rushton's α and my chronaxie, nearer to one or the other, according to fortuitous geometrical conditions. Hence, considering paragraph B above, we see that Lucas's α should not be as unalterable as a full α, nor should it follow physiological changes as closely as a true chronaxie, but should,

as clearly shown by Fig. 7 in my previous article [1933*a*, p. 399], undergo intermediate variations. As a matter of fact, in quite recent researches with electrodes of various sizes, Benoit [1934] finds a more or less marked influence of the temperature according to the size. Moreover, in Lucas and Mines's experiments, one can notice the same gradation. For example [Lucas and Mines, 1907, p. 341], in three experiments, $ET2R$, being 3 or 4σ at the ordinary temperature, is multiplied by 1·6–1·7–1·5, when temperature is lowered 8 degrees. In the subsequent experiments one can calculate $ET2R$ nearly three times longer (*i.e.* nearer to a full σ), and multiplied only by 1·1 or 1·2 for the same decrease in temperature. Benoit has found the same thermic coefficient equal to 2 with stigmatic electrodes and slightly less than 1 with Rushton's block. Thus, Lucas and Mines's figures take their place in the whole series in a logical order.

Taking together all the experimental facts given under paragraphs A, B and C, one must infer that only $ET2R$ given by small electrodes is in accordance with the physiological properties; hence, it is the only chronaxie. Shall I repeat once more that the real meaning of chronaxie is to measure, in the sphere of excitability, a chronological factor governing most, if not the whole, of the vital functions? If any condition, in the practice of electric stimulation, does not give reliable figures in this respect, it is a fault to be avoided by appropriate technics.

I infer that now, partly owing to researches raised by the present controversy, we are in possession of practical rules, theoretically intelligible, for obtaining true chronaxie:

(1) On the nerve, to have an electrode distance of at least 1 cm.

(2) On the muscle, to have a cathode not exceeding 0·5 mm. in size.

Rushton, who for a good while would not admit, as I have already said, the distinction between true and false chronaxie, has, in his last paper, somewhat withdrawn from this position; he says now [Rushton, 1933, p. 359], that if chronaxie is bound to satisfy such rules (I only see the above two, because the "canonical" shape of the curve is not an independent condition; it follows the others), its utility would be limited, whereas "excitation time" would have "great value in physiology." "Excitation time," as we have seen, has no definite sense; Rushton aims at measurement of $ET2R$ made in any manner. This measurement depends at one and the same time on the disposition of electrodes, and on the properties of the tissue; Rushton recognizes this, and even emphasizes it. It would be an error, he says, to suppose that it could be otherwise, except in very particular conditions.

If such were the case, it seems to me that probably chronaxie would not yet have been discovered. Now that we have a clear idea of it, we could again obtain correct figures from such complex experimental data, taking quantitatively into account the influence of electrodes. But it is much simpler to place oneself under those conditions where the chronological

properties of the tissue manifest themselves alone. I can appreciate now how fortunate I have been in working under such favourable conditions, having without special care taken electrodes similar to those used by my predecessors. We must repeat that it is stimulation through Ringer that led to the α error on the muscle, and to another error, which we will point out presently, on the nerve. There are cases where this technique is advantageous, and even necessary, for instance with sartorius; but now we know how to avoid error by taking capillary electrodes not exceeding a given diameter, and in this way we can utilize the classical nerve-free part of the sartorius.

The sartorius has played a special rôle in the history of chronaxie, provoking the use of fluid electrodes, and consequently the production of α curves, because of the rapidity with which, after its excision, its excitability changes, even in a moist atmosphere. This alteration, consisting of a gradual augmentation of chronaxie, soon becomes a total loss of excitability. This latter is however reversible, as Dulière and Horton have shown [1929], by simply washing the muscle abundantly with Ringer. Moreover, a previous bath of Ringer of half an hour's duration prevents the alteration. Before we were aware of this method, we had already obtained correct results, using fine silver electrodes on the muscle exposed, but left in place.

The starting point of this experiment is rather instructive. In our initial researches, as I have said, we had been led to give up this muscle, not, however, without having assigned a larger chronaxie to it than to the gastrocnemius. On revising our former researches [Lapicque and Legendre, 1913] with more precise anatomical determinations, Kreindler [1927] found the fibres supplying the sartorius to be of the same diameter as those supplying the gastrocnemius. If the chronaxie of the sartorius were larger, which law would be wrong, relation between size and chronaxie in nerve fibres, or neuro-muscular isochronism? We remembered that the chronaxie figures obtained on sartorius always increased with time; we used to consider only the first figure of each series; perhaps these were already altered to some degree. And effectively, with the technique I have just pointed out, we [M. Lapicque, 1927; Lapicque and Desoille, 1927] found $0{\cdot}3\sigma$ for the sartorius as well as for the gastrocnemius, on the pelvic as well as on every other part of the muscle. The two laws were thus correct.

Our recent researches, undertaken to study the influence of the size of the electrodes [Lapicque, 1932], on excised sartorii preserved in Ringer, have given perfectly concordant figures, *i.e.* chronaxies of about $0{\cdot}3\sigma$, as long as the diameter of the cathode was between a few hundredths and half a mm. Details of the experiments have been given in my preceding papers in this *Journal*. I will only quote the following conclusion: $0{\cdot}3\sigma$ is certainly the normal muscular chronaxie of the sartorius; locali-

zation of the stimulus on the aneural part of a dissected muscle, stretched out on the bottom of a bath of Ringer, and stimulated by a capillary electrode, can leave no doubt. As for the chronaxie of the corresponding nerve, we had not sought its measure at the time of the above-mentioned experiments, preoccupied as we were with the question of the size of the electrodes. There could be no doubt, however, that it was nearly the same; it is the classical datum for the gastrocnemius, both with direct and indirect excitation; and the fibres of both nerves are, as we have seen, of the same size. On the other hand, the γ excitability in Rushton's experiments as well as in our own, gives a value of this order; and moreover, our stigmatic electrode placed anywhere on this muscle, including the motor point, gives one and the same chronaxie.

Nevertheless, in order to give complete demonstration, we proposed to measure subsequently the chronaxie of the nerve itself simultaneously with that of the muscle.

We came across unforeseen difficulties, which are worth while reporting, as they will serve to clear up an important point of the controversy. It was during last spring, on the frogs *Rana esculenta* or *R. fusca*; on this latter species especially, it is possible, with care, to dissect out the sartorius with its motor nerve; even with frogs of medium size, like those at our disposal, the nerve has a sufficient length for placing electrodes. But stimulation of this nerve turned out to be either wholly ineffective, or, more frequently, effective only a small number of times, so that it was difficult or even impossible to obtain a good measurement. On the other hand, even in our physiological solution, the muscle excitability changed progressively, and its chronaxie increased; in other words, the *modus operandi* of Dulière and Horton [1929], which had succeeded very well in the autumn, was ineffectual. A modification of the composition of our solution would probably be necessary, taking into account recent researches which have given an indication of this. Moreover, the quality of a muscle is subject to considerable seasonal influences which have been brought to light by several authors, more particularly by Wachholder and Nothmann [1931]. In any case, the modification of chronaxie undergone by the muscle was important enough to produce curarization by heterochronism, and to explain the spontaneous disappearance of indirect excitability.

Actually, we have fully succeeded with the Algerian toad, *Bufo pantherinus*, of which Prof. Malmejac kindly sent us some specimens. These experiments aimed specially at verifying the mechanism of curarization; the details will be found in the appropriate paragraph. These animals are particularly favourable both on account of their large size and the stability of their muscular excitability. The same result would probably be obtained on the common toad and even on frogs in the best seasonal conditions and with better adapted solutions. But these experiments on *Bufo pantherinus* are sufficient to establish neuro-muscular isochronism as a direct statement of fact.

Here are some typical figures (chronaxies in farad 10^{-9}; timing resistance, 10,000ω):

		Nerve	Muscle
June 29:	I	50–60	40–50
	II	60	60
July 5:	I	50	75
	II	70	70–80
July 7		55	80–90
	Mean value	58	67

I only see one objection, which perhaps has not been formally given in print by Rushton, but which is implicitly contained in his conception of "γ-like curves": if one could, by diminishing the size of the electrodes, reduce at will the muscle $ET2R$ from α to 0, the value of the nervous chronaxie would necessarily be reached at a certain point, and the equality of both $ET2R$ at this point would have no objective signification. The answer is given above. The reduction of the $ET2R$ with the size of the electrodes does not tend experimentally towards zero, but stops at a size of $\frac{1}{4}$ mm. Any size smaller gives this same $ET2R$, which is no longer a function of the electrode, and this irreducible $ET2R$ is greater the slower the muscle; it increases when the muscle is cooled. It appears as a physiological character; it is the muscular chronaxie.

And now, with the so-called direct stimulation on the usual gastrocnemius, is the muscular substance stimulated directly or by the intermediary of nervous intramuscular twigs?

With small electrodes it is generally admitted that stimulation always reaches the nerve first. I have been a long time hesitating about this. But I am more and more sure that I have often, by chance, with stigmatic electrodes, stimulated the muscular substance itself in the gastrocnemius, and hence got the muscular chronaxie. We can admit this result without denying a greater sensitivity for the nervous twigs; I am now studying more closely the experimental determinism of it, namely, possible paths for electric current through the aneural part of the muscle, but, in the meantime, I find it necessary for the present discussion to report briefly some facts already acquired.

The mechanical appearance of the response may be characteristic, if the local phenomenon is observed with a lens. Having learned on the sartorius to identify muscular response on the one hand, and nervous response on the other, we recognized later both on the gastrocnemius. So as not to describe here the minute particular circumstances of each one, let us say simply that the smallest muscular liminal response can

be so slight as to be doubtful, while the nervous one, if present at all, springs at once into evidence. That is to say, a good number of myones contract "d'emblée"; we have to deal with a peripheral expression of Sherrington's motor unit. By changing the position of the electrodes one can successively obtain each kind of stimulation; chronaxies remain then, on a fully normal muscle, invariable, or nearly so.

I do not intend to put forward this mere equality as a new proof of isochronism; judging from the mechanical appearance of the response, we cannot always be sure enough of its origin, either nervous or muscular. But there are cases where, inversely, chronaxical measurements demonstrate a real muscular stimulation; it is when isochronism progressively breaks down by a spontaneous or toxic alteration of the muscle, and passes on to a sufficient isochronism for stopping indirect excitability. Thus two approximately equal chronaxies at the outset characterize themselves by their evolution; one of them remains practically invariable until it disappears, while the successive figures of the other, with the time as abscissa, trace a continuous curve even after the nervous stimulation has become ineffective. This second chronaxie can thus only be muscular chronaxie.

Such cases are of great importance in the present state of the theory of curarization, especially for the present discussion; we will give further on every necessary experimental precision. I considered it desirable to give in advance the preceding explanations with a view to avoiding in this experiment a possibly paradoxical aspect.

Until last year the discussion about chronaxie was limited to muscular excitability. In 1923 Davis stated that all experiments gave concording figures for the nerve. Even in 1932 Rushton pointed out the "striking difference" in this respect between nerve and muscle; moreover, he found it necessary to elaborate an explanation, because this fact was contradictory to his own theory. So he came to the fantastic conception of the myelin sheath as an insulating medium; the current would only penetrate by faults in this sheath, namely Ranvier's nodes, the size of these faults being thus the actual electrode factor. He gives no justification at all for this suggestion, except that it is evident "on histological grounds" [Rushton, 1932, p. 469].

I should very much like to know of any histological evidence for attributing to any tissue greater or smaller electrical conductivity. In any case, the classical theory of electrotonus implies for the myelin a real conductivity, although smaller than for the axon; it implies also that the current reaches the latter by crossing the myelin. But we have

already seen that Rushton seems to be ignorant of the significance of electrotonus.

But very soon after, Grundfest [1932] published a paper in which he stated that, contrary to current opinion, he found, with very fine electrodes applied directly to the nerve fibre, $ET2R$ very much smaller than with ordinary electrodes applied to the nerve trunk, 0·024–0·08σ, while the same electrode applied to muscle fibre gave values only slightly below the usual ones.

Rushton [1933] judges this result as "of the first importance" against isochronism. Indeed, apparently, with any one and the same electrode, whether large as in Rushton's experiments, or very fine as in Grundfest's, $ET2R$ for the muscle is always greater than for the nerve. We could ask Rushton now what are we to think of the above-quoted theory of Ranvier's nodes as timing factors for nervous electrical stimulation? But we have more objective criticism to raise.

Grundfest, following other physiologists, utilizes the frog's retro-lingual membrane where single muscle and nerve fibres are naturally separated; the preparation is immersed in Ringer; the anode is diffuse in this bath, and the cathode is made of a glass tube simply pulled out in a microflame to a capillary size with an opening of about 20–60μ in diameter; this tube is filled with Ringer and dipped in the solution down to the fibres. In these conditions the effective factor on the nerve is not the size of one electrode, but the very small distance between the two; the current enters (in the common conventional meaning) the tissue in the immediate neighbourhood of the capillary wall; here is the actual anode, separated from the cathode only by the thickness of this wall. Conforming to the known law for the nerve, in virtue of electrotonus, $ET2R$ is reduced a good deal below chronaxie. Once again, the Ringer bath has given rise to a false chronaxie.

Under the same conditions, Sakamoto [1933], without being aware of this, found a very similar fact on macroscopic nerves, *e.g.* the lumbar nerves of the frog, by covering his preparation, possibly to avoid desiccation, with a Ringer bath in which he plunged a diffuse anode; his cathode was a glass electrode, obtained by blowing out a bulb at the end of a 3 mm. tube and then grinding it so as to obtain a minute pore of 9μ in diameter. The conditions were thus similar to Grundfest's, but the plane obtained on the bulb by grinding being applied to the tissue, the distance between the orifice and the nearest point where the tissue is in contact with the outside liquid serving as an anode, is of the order of the millimetre, that is to say, much larger than with Grundfest's

electrode. Besides, there were passive tissues interposed, of various thickness, but evidently always greater than in the experiment on a single fibre. For these reasons the error must be smaller. In fact, Sakamoto, for nerves whose classical chronaxie was probably about 0·3σ, finds "chronaxies" varying between 0·04 and 0·2σ, an average of 0·11σ, *i.e.* three times smaller; Grundfest's figures, on the average, are three times smaller still, that is to say their error is three times greater than the Sakamoto error, on account of the thinness of the wall of the electrode and the more exact application of the latter.

This comparison answers perfectly the theoretical conjectures. One peculiarity of Grundfest's results (emphasized by him), namely that "the voltage-capacity curves of muscle and nerve fibres are different in shape," is further explained on these grounds. I have shown long ago [Lapicque, 1926, p. 236] that the reciprocal reaction of two electrodes of opposite sign is bound to give a systematic deformation of the curve I had not yet called canonical. This deformation is precisely what appears in Grundfest's curves relative to the nerve. It implies a rise of the threshold chiefly for long durations. Now, Grundfest's figures show higher rheobases for nerve than for muscle—a fact quite contrary to the usual rule, but easily explained by the deformation of the curve alluded to above.

In any case, it is easy to show experimentally the rôle of the Ringer bath by taking measurements of *ET2R* on a given nerve, sometimes "à sec," sometimes under Ringer.

The following are the figures obtained under these conditions on the lumbar plexus of *Rana fusca*. The animal prepared *à la Galvani* was fixed with the ventral face uppermost, on the bottom of a porcelain basin coated with paraffin. The capillary cathode was placed on a nerve by means of a stative easy to regulate with precision, a silver plate forming the anode in contact with the trunk muscles. After a measurement "à sec," it was easy, without disturbing the contact, to cover the preparation with physiological saline, and then to remove this by means of a siphon. The internal diameter of the cathode was 0·4 mm., the thickness of the wall 0·8 mm.; stimulation was delivered by means of a condenser discharge with a shunt ensuring invariably 10,000 ohms as the reduced resistance; among the several muscles stimulated simultaneously with this procedure, the liminal twitch was observed on any one arbitrarily chosen in order to test excitability of the corresponding nerve fibres; the muscle observed in each case is specified below; the figure given expresses in farad 10^{-9} the liminal capacity under a potential twice the rheobasic value.

(1) "À sec": on the tibial 65, on the superficial flexor 80–85; immersed in Ringer: tibial 32, flexor 31.

(2) On the flexor of a toe, nerve "à sec": 90–100; the same, under Ringer, 35; solution drawn off 70.

(3) On the sartorius: under saline 30; solution drawn off 48–50; dried with cotton wool in the neighbourhood of the preparation and left to drain 57, then 65.

I then took another electrode, of the same internal diameter, but with a thinner wall.

(4) Superficial flexor nerve: under saline 35; after rapid drawing off of solution 38; after drying as above 70.

(5) Experiment on a smaller and specialised nerve (twig innervating the semimembranosus): under saline 27; after drawing off of solution 57.

These figures suffice to show objectively the rôle of the electrolyte bath surrounding the capillary electrode; this has produced in my experiment exactly the same small values observed by Sakamoto ($30 \cdot 10^{-9} \times 10^4 \times 0 \cdot 37 = 0 \cdot 11 \cdot 10^{-9}$). The influence of the thickness of the wall hardly appears, probably because of the thickness of the interposed tissues.

Grundfest's still lower figures are perfectly explained by the special anatomical conditions coupled with the much greater fineness of his electrode. This author has made very few experiments on preparations "not covered with Ringer"; he seems to consider them as showing that his results cannot be put down to the influence of the solution. But, firstly, the values so obtained are notably increased, being double the preceding ones; and secondly, some of my above results show that a shallow layer of liquid still constitutes a cause for a very important error; the presence of such a layer is not excluded in Grundfest's experiments.

To sum up, the stimulation of nerve under Ringer by means of a capillary electrode gives a false chronaxie, the error, easy to demonstrate experimentally, being clearly explained by the known influence of electrotonus when one has two electrodes close to one another. This effect is not observable on the muscle, or if so, only to a much smaller degree. I have demonstrated the distance of electrodes to be relatively unimportant on the muscle [Lapicque, 1932, p. 266].

Consequently the fact of finding, by means of this technique, different values for the muscle on the one hand, and for the nerve on the other, cannot be invoked against neuro-muscular isochronism.

We can summarize in a schematic graph (Fig. 1) the aberrations introduced in chronaxie measurements by defective conditions of the electrodes. On the nerve, with electrodes of any size, but not brought nearer to one another than 10 mm., and on the muscle, with electrodes at any distance, but with a cathode not exceeding 0·5 mm. in diameter, we find one and the same chronaxie.

There is no difficulty in realizing at the same time both conditions. Except perhaps for smaller distance between electrodes this has been the case with the most ordinary physiological apparatus for electrical stimulation. Under these conditions neuro-muscular isochronism appears *de plano*. The chronaxie, common to both nerve and muscle, is figured in XY.

But, if a fluid cathode not restricted in size is used, there is no or little change for the nerve, whereas for the muscle $ET2R$ rises above chronaxie, more and more as the diameter increases above 0·5 mm. Hence Lucas's or Rushton's heterochronism is obtained, figured in kl on the right-hand side of the graph.

If the two electrodes are brought nearer to one another than 10 mm., or else, what is practically, as we have just seen, the same, if a capillary tube is taken as cathode and immersed in a Ringer bath as anode, little or no change is observed for the muscle, whereas for the nerve *ET2R* becomes shorter than chronaxie, shorter and shorter as the distance decreases. Consequently, Grundfest's heterochronism is obtained, as figured in *fg* on the left part of the graph.

Both heterochronisms are simply artefacts.

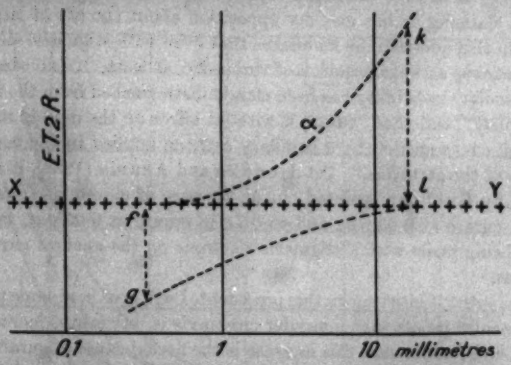

Fig. 1. Schematic graph, showing how either nervous or muscular chronaxie measurements can be altered by faulty electrode conditions (see text). Diameter of cathode, or alternately distance between electrodes, as abscissa on a logarithmic scale.

Rushton's fundamental argument against our theory of curarization is that the α curve is not modified by curare.

So we are in the presence of the following dilemma: either *ET2R* derived from this curve is not muscular chronaxie, or else muscular chronaxie remains unchanged by curare. The increase of chronaxie by curare seemed to me a fact so obvious, so often observed, and so easy to demonstrate, that, at the beginning of the present controversy, having myself stated that curare does not change α, I had published the fact with a certain satisfaction, as an argument against the physiological signification of α [Lapicque, 1931*b*].

In his last paper [1933] Rushton, who had summarily pointed out the same fact in 1930 [Rushton, 1930], devotes seven pages to this, multiplying proofs and examples. Perhaps it was not necessary to insist in

such a way on the demonstration of a single experimental point admitted on both sides. The question was to solve the above dilemma.

A few months later it was shown that α remains constant on a quick or a slow muscle [Lapicque, 1933*a*], or when a muscle is cooled [Benoit, 1933]; consequently, if it does not change after curarization, this cannot constitute an argument against the idea of an increase of chronaxie by curare. Perhaps Rushton, if he had been cognizant of these facts, would not have manifested such contempt for my previous arguments as to have dismissed them in three lines of text.

But claiming he has met my opposition about the use of large electrodes in this question, he maintains that even with stigmatic electrodes there is never an augmentation of chronaxie, at least, "in no case where the muscular excitability has been clearly distinguished from the nervous excitability," and that "curare is without effect on the muscle strength-duration curve elicited by a capillary cathode applied to the nerve-free portion of the sartorius." Yet Jinnaka and Azuma [1922], in a paper quoted by Rushton, pointed out an increase of chronaxie from 0·3 to 0·5σ in curare at 0·03 p.c., and to 0·7σ in curare at 0·05 p.c., measurements being made with a stigmatic electrode on the aneural part of the sartorius.

But, without insisting on this precedent, I will give here some personal experimental proofs that muscular chronaxie is, in reality, increased by curare, and further that this increase is the actual cause of curarization; we will then examine the arguments upon which Rushton has based his drastic opinion.

Already in some of the experiments upon which 25 years ago we founded our theory, it is possible to follow a gradual increase of a chronaxie, unquestionably the muscular one, since it follows its own evolution after the indirect excitability has disappeared.

These experiments have never been published before in full; previous to chronaxie, they consisted in determining strength-capacity curves from which we deduced a parameter more or less exactly equivalent to the chronaxie. This circumstance permits a fuller appreciation than the measurements reduced to direct determinations of chronaxie.

May 17, 1906. *Bufo vulgaris*. Stimulation of the gastrocnemius by two metal wires stuck in the muscle. $R = 22{,}500\omega +$ the muscle, say about $25{,}000\omega$. The sciatic is prepared to test indirect excitability. At 3.10 p.m. 1 mg. curare is injected; at 3.25 the muscle still responds to an induction shock on the nerve; at 4.20 even the strongest possible

repeated shocks have no effect. The following are the figures for the strength-capacity curves at various times:

	Time				
Capacity × 10^6	2.35 p.m.	3.0 p.m.	3.20 p.m.	5.0 p.m.	5.40 p.m.
50	1·2	1·2	1·9	1·8	1·8
20	1·45	1·45	2·4	2·35	2·35
10	1·80	1·85	3·0	3·25	3·2
5	2·0	2·2	4·0	4·6	4·4
3	—	—	—	—	6·05
2	3·1	3·2	6·8	>8	—
1	4·5	4·7	—	—	—

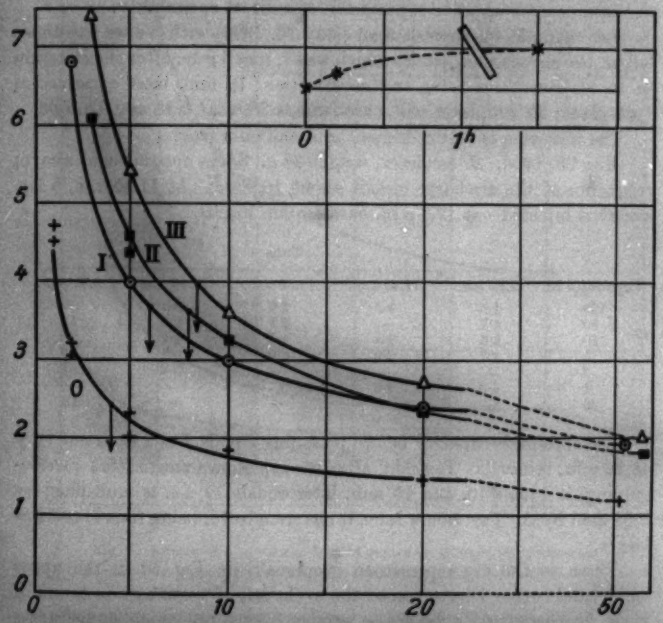

Fig. 2. Strength-capacity curves on a toad's gastrocnemius; 0, before curare; I, II, III, after a small dose of curare. In the upper right-hand corner, variation of the chronaxie with the time in hours as abscissa.

Curves expressing these figures are reproduced in Fig. 2. Curve 0 represents excitability before curare (a chronaxie-capacity $Cw=4$ can

be read there). Curve I (3.20 p.m. column) represents excitability during the time while the action of the poison is evolving, curarization is not effective: $Cw = 6$. Curve II represents excitability after curarization is complete (5.0 p.m. and 5.40 p.m. columns, practically identical): $Cw = 8$. Twenty-four hours later, thresholds are hardly raised at all: $Cw = 7$ or 9. We have here a progressive evolution of direct chronaxie, with suppression of indirect excitability when chronaxie has doubled its value. In the present case the evolution stops here, because the dose has just been sufficient to produce curarization in no less than an hour (the toad is less sensitive to curare than the frog). When the dose is above this limit, evolution continues after suppression of indirect excitability.

For example, on another toad (May 19, 1906) with a dose five times larger, the chronaxie-capacity which was 7, rose 5 min. after the injection to 10 without there being any curarization; 10 min. later curarization took place; 20 min. later still, chronaxie is 38, that is to say five-fold.

The following is an experiment in detail on a frog.

May 28, 1906. *R. esculenta*, weight 28 g. Same apparatus as above; resistance of the discharge circuit about 10,000ω. At 11.45 a.m. 5 mg. curare is injected—at 12.5 p.m. curarization begins.

	Time				
Capacity $\times 10^2$	11.20 a.m.	11.40 a.m.	11.55 a.m.	12.10 p.m.	2.30 p.m.
100	1·5	1·5	2·0	2·0	9
50	1·7	—	2·5	2·4	12
10	2·3	2·4	4·1	4·4	25
5	2·7	2·9	6·0	6·6	36
3	—	—	—	—	51
2	4·6	4·6	>8	—	—
20	—	—	—	—	16

The chronaxie-capacity before poisoning equals 4 and does not vary at 20 min. intervals. Ten min. after the injection (curarization just beginning) it equals 10, and 15 min. later equals 12, *i.e.* is multiplied by 2·5, then by 3. Two hours later it has risen to 16, being thus four times larger.

These results are represented graphically in Fig. 3; in the upper right-hand corner I have traced the evolution of chronaxie in terms of time of poisoning; it proves a regular curve, on which curarization appears at a certain point without interrupting its continuity. How can we understand such results without attributing the measured chronaxies to muscle substance?

There are, nevertheless, very frequent cases where the so-called direct stimulation actually reaches the nerve fibres; then one actually sees the

chronaxie passing abruptly from a given value to a value about double at the moment when curarization is produced. But this case, if not as demonstrative as the preceding one, is quite easy to reconcile with it.

A great many observations of this kind have contributed to give birth to our theory, which during a quarter of a century has received numerous

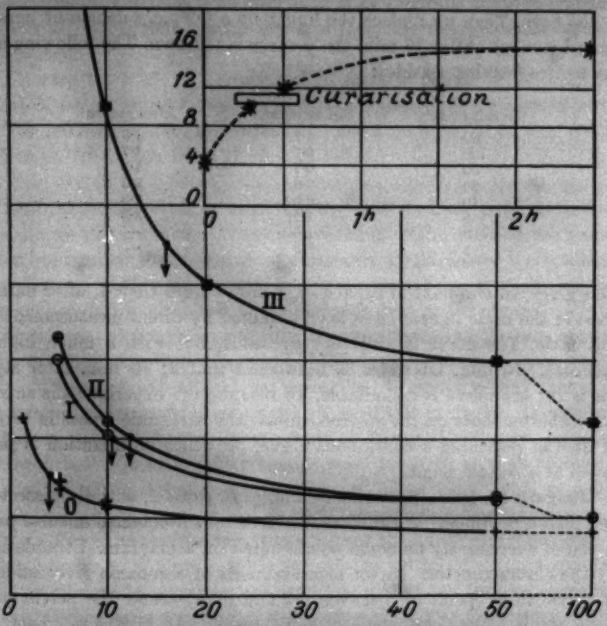

Fig. 3. Effects of a medium dose of curare on a frog's gastrocnemius, pictured in the same manner as in Fig. 2.

confirmations from many workers, on various animals, with various poisons. However, in order to meet directly a negation raised by Rushton in the Physiological Congress in Rome, we have taken up recently the action of curare on sartorius.

November 28, 1932. Sartorius dissected at its pelvic end, but left in contact with the muscular mass of the thigh in the neighbourhood of the entry of its nerve; on the other hand, the lumbar nerves were

dissected, and separated from the cord, but left in continuity with the thigh, which is severed from the body and freed from the leg. The whole is immersed in Ringer; and the aneural part of the sartorius being drawn aside as much as possible, a capillary electrode is applied to it. We first find, with condensers, an α and a γ curve, depending on whether the capillary electrode is anode or cathode, the other electrode being diffused in the bath. Then we replace the liquid by a 0·2 p.c. solution of curare (gourd curare). After 15 min. the γ curve is redrawn. The following are the figures (leaving α aside):

Capacity $\times 10^6$	Before curare	After curare
200	0·7	1·5
100	0·7	1·7
50	0·7	1·9
20	0·8	2·2
10	0·9	2·8
9	1·2	3·3
3	1·6	—
1	2·3	—

This gives, as chronaxical capacities, before curare $Cw = 4$, after curare $Cw = 7$; the same figures have been obtained by direct measurement of chronaxie. The nerve is still very excitable, but with a much higher rheobase. 10 min. later, Cw is between 8 and 9; 10 min. later still, Cw is 10; the nerve is inexcitable. To resume, the experiment is superimposable on those on the gastrocnemius; the chronaxie traces in terms of time of poisoning a continuous curve, on which curarization is produced at a certain point.

January 12, 1933. Sartorius of a large *R. fusca* ♀, entirely dissected and placed in Ringer. A capillary electrode on the classical aneural part is placed successively on some points noted on a diagram. Stimulation by the chronaximeter. Direct measurements of chronaxie give: middle of pelvic third (point a), between 0·2 and 0·3σ; nearer the pelvic end, same result. Again on point a, same result. At 11.25 a.m., bath of curare (0·2 p.c. ticuna curare). We then determine strength-duration curves for the point a.

	Time		
Time in σ	11.50 a.m.	12.15 p.m.	2.10 p.m.
100	2·5	9·0	1·9
20	2·6	9·2	2·0
10	2·6	9·2	2·1
5	2·6	9·4	2·2
3	2·7	11	—
1	3·5	13	3·6
0·5	4·7	17	6·0
0·3	5·9	—	—

After the action of the curare has lasted 25 min., the chronaxie has a value of 0·5σ (almost doubled), and after 2 hours is 0·9σ (more than tripled). The progressive augmentation is manifest. The moment of curarization has not been observed, but at the end of the experiment the stigmatic cathode placed on the motor point gave a chronaxie of 0·8σ, very nearly the same as on the aneural part; curarization was thus complete. The muscle treated as in May's method shows the noted point *a* of stimulation a good distance from the nearest visible nerve ramification.

January 17, 1933. A sartorius of *R. fusca* is placed (11.45 a.m.) in a bath of 1 p.c. curare; a capillary electrode is placed on the aneural part.

The following values are obtained: 11.48 a.m. 0·4σ; 11.55 a.m. 0·8σ; 11.59 a.m. 1·4σ, then 1·8σ; 12.10 p.m. 4–5σ.

Such experiments show incontestably that we have put into action a substance which augments muscular chronaxie. The question has sometimes been raised if the change of chronaxie would not be a secondary phenomenon, not even due to the curarizing substance itself, but to another substance contained in the complex drug called curare.

Rushton, although repudiating the phenomenon in the case of just sufficient curarizing doses, has applied this interpretation to cases where it would be produced with higher doses; he has even specified that it is the mineral substances of curare which have this property. In support of this explanation he quotes an article of Mines [1908], who, he says, "showed that the excitability change was the same as that produced by the ash of an equal quantity of drug" [Rushton, 1933, p. 350]. But Mines's observation (which in itself is very interesting) is concerned with a different phenomenon. It is a case of the automatic contractions which appear in a muscle subjected to the action of a pure sodium salt, not counterbalanced by calcium. The rôle of the nervous elements in this phenomenon was and is still discussed; it was interesting, then, to know if curarization would put an end to this phenomenon. Mines stated that it had no hand in the matter and that his own curare only had an effect with relatively strong doses, the calcium supplied with the curare being then abundant enough to balance the sodium. This mechanism is very clear, but quite apart from our problem. Rushton has quoted the result of this experiment under the ambiguous form "excitability change," which could apply to both questions, but Mines's experiments with curare ash are concerned only with the question of spontaneous excitation. In any case, the opinion that calcium in the

curare is the cause of augmentation of chronaxie having been expressed at different times, I thought it better to make special experiments to verify this point.

Already, some years ago, I had precipitated this ion by sodium oxalate in a solution of my curare, and proved that the augmentation of chronaxie remained the same. (This control has not been published.) Recently, I wished to study quantitatively this influence of calcium. I have stated that, on a normal muscle, it is practically nil up to doses much larger than that which curare can bring, though in very strong doses, *e.g.* in pure isotonic solution, it increases chronaxie [Lapicque and Nattan-Larrier, 1926]. The dose of the calcium of Ringer can be tripled without observing any systematic effect. A frog which receives an injection of 3 mg. of calcium (chloride) does not manifest any disturbance, and gives normal muscular and nervous chronaxies at the end of an hour, without any change compared with the measurements taken before the injection. The dose of curare corresponding to this figure, whilst admitting an amount of calcium as high as 5 p.c., should be 60 mg.; it is higher than any dose I have ever worked with. A weight of curare simply equal to the weight of calcium injected would increase the chronaxie ten-fold. Thus calcium does not take the slightest part in the observed change.

And if it did so, that would not constitute an argument against the chronological theory of curarization.

Rushton, thinking to confirm Mines's statement about calcium, quotes another statement of Jinnaka and Azuma about KCl increasing chronaxie. Actually, KCl does so, at least in sufficient dose, and in doing so, it curarizes, like any substance that increases chronaxie [Nattan-Larrier, 1928]. Subsequently, Reiss [1930] found it logical to range potassium in a pharmacological series with curarizing amines amongst which the active substance itself of curare is chemically classified by Boehm. The minute quantities which may exist in the different kinds of curare I know of are not likely to exert any such effect, and even, in small doses, potassium acts in the reverse sense; but it matters little if curarization is the algebraical sum of mineral and organic substances. The most effective factor for curarization is the change of chronaxie.

For instance, unbalanced NaCl, *i.e.* isotonic solution of NaCl without calcium, curarizes, as we know since 1894 [Locke], and a small addition of calcium re-establishes indirect excitability. Well, under such condition, NaCl increases muscular chronaxie, measured on the aneural part of a

sartorius, a statement quite in agreement with a change of excitability noted by Mines; moreover, the addition of calcium reduces chronaxie nearly to its original value, and thus re-establishes indirect excitability [L. and M. Lapicque, 1933*a*].

On the other hand, there are definite organic substances, possessing pure crystallized salts, which curarize, as curare, by raising the muscular chronaxie, for example, sparteine [Weill, 1913]. With such poisons, which have no mineral impurity, one can observe, exactly as in curarization by curare, a gradual increase of muscular chronaxie, and separation between nerve and muscle linked with a sufficient degree of heterochronism.

I will report a few unpublished experiments made recently with Madame Lapicque on sparteine, following the same plan as in the above experiments on curare.

January 21, 1933. Sartorius of *R. fusca* ♂, carefully dissected, placed in a bath of Ringer at 4.14 p.m. with 0·1 p.c. sparteine sulphate. Capillary electrode 0·4 mm. diameter placed invariably on the aneural part; measurements of chronaxie with the chronaximeter.

Time of action min.	Rheobase	Chronaxie
5	3	0·6
12	2·3	0·9
18	2·1	0·8
23	1·9	1·1
28	2·2	1·3
35	3·2	2·0

At the 23rd minute the form of the response had appreciably changed; curarization had probably just occurred.

January 24. Same arrangement; bath of 0·2 p.c. sparteine.

Time of action min.	Rheobase	Chronaxie
9	2·5	0·7
12	1·8	0·8
25	4·0	1·3
31	4·3	1·7

At this moment the electrode was placed on the middle of the muscle (motor point); we find as rheobase 3·4; chronaxie 1·9; these figures, being the same as for the aneural part, show that there is no longer any nervous excitability. The aneural part gives then (40th minute) a chronaxie of 1·9.

January 21. Bath of 0·4 p.c. sparteine; same arrangement.

Time of action min.	Rheobase	Chronaxie σ
6	6·0	1·3
8	5·6	2·0
20	8·5	12

We see that, with a much stronger dose, chronaxie rises to a much higher value.

It seems to me we have amply verified that curare and various other curarizing substances augment muscular chronaxie, even in cases where muscular excitability is unquestionably distinguished from nervous excitability. Further, a series of facts show that this augmentation is the essential phenomenon; for curarization, in the classical sense, *i.e.* disconnecting of the nerve, "décrochement" as Claude Bernard calls it, intervenes during the process without influencing it; thus it is the effect and not the cause of the augmentation of chronaxie.

We have obtained quite recently the most direct demonstration of this in observing simultaneously on *Bufo pantherinus* the muscular chronaxie on the aneural part of the sartorius and the chronaxie of its motor nerve during the course of the poisoning. The following are some of the experiments.

June 29, 1933. *Bufo pantherinus*, weight 90 g. Sartorius dissected leaving a good length of its own nerve which is placed on my electrodes with ebonite cover; a capillary electrode is placed on the muscle (aneural part) which is left dry. Measurements of chronaxie by condensers in farads 10^{-9}. Shunt giving very nearly the same resistance ($10{,}000\omega$) for the discharge circuit in both cases.

We find, in the fresh state: nerve—rheobase = 2·8, chronaxie capacity, $Cw = 60$; muscle—$R = 2{\cdot}2$, $Cw = 60$. (This is one of the direct verifications of isochronism already reported.)

At 2.14 p.m. the muscle is moistened with a solution of 0·15 p.c. curare in Ringer. 5–10 min. after, we find: nerve—$R = 5{\cdot}1$, $Cw = 50$; muscle—$R = 3{\cdot}3$, $Cw = 80$.

The muscle is moistened again and covered with a little hydrophilous cotton wool soaked in the same solution.

We find, at 2.45 p.m.: nerve—$R = 7$, $Cw = 55$; muscle—$R = 1{\cdot}6$, $Cw = 120$. At 2.53 p.m.: nerve—$R = 9$; muscle—$R = 1{\cdot}4$, $Cw = 120$.

At 3.5 p.m. the muscle is moistened again with 1 p.c. curare. Almost immediately after: muscle—$R = 1{\cdot}2$, $Cw = 135$, then 140.

The nerve is still excitable. It ceases to respond at 3.40 p.m. We then find for the muscle: $R = 1{\cdot}2$, $Cw = 150$, then 160.

No more change until 4.15 p.m.

We then wash the muscle with Ringer without curare. At 4.22 p.m.: $R=1\cdot3$, $Cw=140$–150.

The washing with Ringer is repeated abundantly. The nerve reappears to be feebly excitable. We find, for the muscle, $R=3\cdot7$, $Cw=90$.

July 3, 1933. *Bufo pantherinus* ♀, large size. The two sartorii with their nerves are dissected. The two muscles "à sec" give respectively chronaxies of 61 and 55. Both are then immersed in a bath of Ringer. After a few minutes both have a chronaxie of 30. One, *A*, is left in Ringer; the other, *B*, is placed, at 4.10 p.m., in the same solution with the addition of 0·5 p.c. gourd curare.

At 4.20 p.m. the preparation is taken out of the bath for stimulation "à sec" as in the preceding experiments. The rheobase of the nerve, which was 2·5 when taken out of the Ringer bath, has risen to 10. On trying to measure its chronaxie, indirect excitability disappears. The muscle gives $R=2\cdot5$, $Cw=150$. At 4.30 p.m. we find for the muscle $R=3\cdot8$, $Cw=240$. It is replaced in the bath of curare, where, in 3 hours, its chronaxie rises to 600.

A gastrocnemius of the same toad is dissected with its sciatic. We find "à sec": nerve—$R=1\cdot1$, $Cw=70$; muscle—$R=2\cdot0$, $Cw=65$. The preparation is placed in the bath of 0·5 p.c. curare at 4.50 p.m.

At 4.55 p.m.: nerve—$R=1\cdot0$, $Cw=65$; muscle—$R=1\cdot2$, $Cw=40$.

At 5.15 p.m. the nerve rheobase rises to 4·5; direct stimulation gives $R=1\cdot5$, $Cw=90$–100.

At 5.25 p.m. the nerve is almost inexcitable; even a strong stimulus provokes in the muscle only a feeble localized contraction, which does not increase either in extension or in strength with the increase of the intensity of the stimulus; that is to say, the most part of the myones are curarized. Direct stimulation gives $R=2\cdot4$, $Cw=110$. The preparation is replaced in the bath of curare. At about 7 p.m. the muscular chronaxie has risen to 400.

At 6.10 p.m. the sartorius *A*, which has remained in the Ringer, gives: nerve—$R=1\cdot5$; muscle—$R=2\cdot2$, $Cw=55$.

At 6.15 p.m. it is placed in a bath of curare like the other muscles. After 10 min. the rheobase of the nerve has risen to 10–12; the chronaxie of the muscle to 100. After 15 min. the nerve is still inexcitable, the chronaxie of the muscle rises to 120, then to 150; its rheobase has not appreciably changed. Left in the bath of curare until 7.15 p.m., its rheobase is found to be 3·3, its chronaxie 250 or 300.

The evolution of chronaxies according to the time of the action of

the poison is shown graphically in Fig. 4. The point where indirect excitability disappeared, marked out by a rectangle crossing the curve, is without the slightest effect on its continuity; on the other hand, the position of this sign of curarization at nearly the same height on each

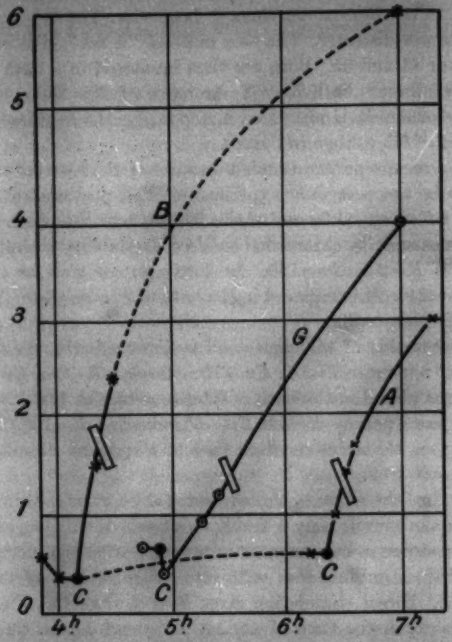

Fig. 4. Variation of chronaxie according to the time in hours, for several muscles of *Bufo pantherinus*. On each curve the intervention of curare is marked out by a black dot, and curarization by an oblique rectangle.

curve is to be noted; this height indicates the upper limit of heterochronism allowing the excitation to still pass from nerve to muscle.

How can Rushton have denied the existence of a phenomenon so obvious as the increase of chronaxie by curare? I have offered to show it to him several times. I regret that he has never been able to visit my laboratory; that would have saved a good deal of paper and

ink. He seems to have been hypnotized by the α curve, whose value he thought he had established as a standard for muscular excitability, and which refused to vary with curarization.

In his historical summary he devotes 20 lines of print to Jinnaka and Azuma's experiment on this point without letting the reader know of the unquestionable increase of muscular chronaxie reported by these authors, a fact which I have quoted above (p. 126). He does not even mention the capital fact discovered by Brücke [1868] and universally accepted since that time, namely, a slower excitability appearing in direct stimulation after curare. Amongst the countless physiologists having made this same observation, he quotes only Boehm, and even then in a strange manner; he asserts Boehm to be in agreement with Watts and with Grundfest; but Watts and Grundfest claimed "that curare has no effect whatever upon the excitability of muscle" while Boehm [1910] stated formally an increase of chronaxie, adding, it is true, that curare does not really increase muscular chronaxie, but simply reveals the normally slow muscular excitability.

I wondered why Rushton was systematically silent about this old classical opinion, for it seemed to be in accordance with his own conception of the α excitability. But we can notice that all the classical experiments have been performed with stigmatic electrodes, and now Rushton denies that the long muscular chronaxie can be detected in this manner. Thus, he stands in opposition to the classical theory as well as to my own. The only authors that can be quoted in agreement with Rushton's views are Watts and Grundfest. The singular results they report must have been obtained under particular conditions difficult to determine.

Watts has published very summary observations; at first I did not understand them at all, that is why I did not mention them in 1926. Grundfest does not give any more details on this point. However, we can remark that these two authors have one thing in common, which distinguishes them from others, that is stimulation under Ringer. This condition does not absolutely prevent the phenomenon, as can be seen in our above experiments, but I am disposed to admit that it introduces some complication. If we take from a frog, curarized by injection, a muscle whose chronaxie is considerably increased, and plunge it into Ringer, its chronaxie diminishes; the same can happen for a muscle treated first with a solution of curare, and then washed with Ringer. In spite of this diminution of heterochronism, indirect excitability is not often restored, but, on the other hand, rheobase has risen considerably.

I think it necessary, in a second approximation, to take into account rheobase side by side with chronaxie in curarization. This point of view fits in entirely with the theoretical explanation of curarisation by heterochronism, but that is a matter for another paper.

In any case, if Ringer brings in any complication whose clearing up demands new work, this is simply a detail outside of any physiological conditions.

The very few and summarily reported experiments worked out by Rushton himself are concerned with the aneural part of the sartorius, but they are very likely liable to the following error. As I have pointed out, p. 119, sartorius, in spring, is subject to changes in excitability, even in Ringer; its chronaxie rises and indirect excitability disappears spontaneously. Either with such spontaneous alteration, or with curarization with any poison, when sufficient heterochronism is reached, one would still often obtain a response on stimulating the nerve, but, as I have reported above, this stimulation becomes ineffectual after a very few trials.

That seems to have happened with Rushton's sartorii, for it appears in his figures that chronaxie has nearly doubled, spontaneously, when he adds curare. The dose of curare added is an extraordinarily small one, just a third of the minimum indicated by Lucas for the same laboratory curare; nevertheless, indirect excitability disappears after an incredibly short while, with a very slight, if any, increase of muscle chronaxie.

The question is if curare intervenes here at all in curarization, and if without its addition the same thing would not have happened. It is stated in supplementary information furnished by Rushton through the kind intervention of Prof. Adrian, that: (1) the above-mentioned experiments were only seven in number, each based on one and the same programme; (2) all these experiments were performed in June and July; (3) only one trial of indirect excitability was made before adding curare; (4) from this moment on, trial was repeated every minute until it appeared ineffectual; (5) no experiment of control without curare was ever made.

In such experiments nothing prevents anyone acquainted with the behaviour of the frog sartorius in spring to consider Rushton's results as fully agreeing with my theory, *i.e.* neuro-muscular disconnection by heterochronism; as a matter of fact, this heterochronism is stated by Rushton as having reached the required degree, or nearly so; curare brought no or little extra change, because its concentration was too small and the time allowed for its action too short.

Consequently, Rushton has no longer any unequivocal evidence to raise against the large number of experiments demonstrating, first, that isochronism normally exists; secondly, that curare increases muscular chronaxie; thirdly, that neuro-muscular disconnection follows hetero-chronism.

Thus, the whole part of the theory is justified; indeed the points quoted are not merely a "first argument," as Rushton calls them; they are the theory itself.

Two other arguments arbitrarily chosen for criticism amongst many others are only secondary, and they can be summarily dealt with.

I had used progressive currents, less in order to test curarization itself than as an indirect proof of isochronism before curare [Lapicque, 1926, p. 246]. Since we have now direct proof, this indirect one has lost much of its interest; but it remains correct. I have already, in my preceding paper [Lapicque, 1933*a*, p. 402], explained what surprising ignorance of the question Rushton has displayed. I will not insist on the point. I will simply say that his own experiments do not afford any contradictory fact. The experiment is the following. A sartorius, left in the Ringer for an hour or more, becomes less sensitive to an abrupt current and more sensitive to a slowly increasing current. Curare is then added (always in the same very small proportion, I suppose); indirect excitability being abolished in a few minutes, the sensitivity to various slopes of current shows hardly any new appreciable change. Rushton this time has made no measurements of chronaxie. But this series of experiments having been carried out under the same conditions as those exposed in the preceding paragraph, the muscle must have undergone, in the same way, an increase of chronaxie before curare and little or no change in the following minutes. Then, the action of progressive current is precisely what it ought to be, according to the law which Rushton has mocked as "a bold assumption."

The last point that Rushton contests is antagonism between veratrine and strychnine. As he remarks, with reason, if the theory were not previously founded on other grounds, we would probably not have tried it; it appears thus, as a striking counterproof of the theory which has led to its anticipation. But, having made a few experiments without succeeding in observing it, Rushton claims to have demonstrated that "strychnine and veratrine do not antagonize each other." I do not wish to insist upon the discourteous manner shown in denying without reserve

a fact, a material fact, which I have affirmed to have seen, but I must point out his formally expressed contempt of the rule that a negative statement is unable to cancel a contrary positive one. He has not even seemed anxious to explain how I could have been led into the error in observation which he blames me. Well, I intend here to give new evidence that strychnine and veratrine do antagonize each other and then to point out the reasons for which Rushton has probably failed in his own experiment.

I have recently, at the *Congrès des Physiologistes de langue française à Liège* [Lapicque, 1933*b*], explained in detail how interesting for the chronological theory are the various antagonisms in curarization. For instance, physostigmine, emphasized by Rothberger [1901 and 1902] as an antidote for curare poisoning, showed that it acts by diminishing muscular chronaxie [L. and M. Lapicque, 1912]. It even happens that in this sense it curarizes by heterochronism contrarywise to that of curare. Such a strange fact of two poisons, each one being able to curarize if given alone, but one curing curarization produced by the other, is explained very simply by our theory; it is a case of two opposed actions on a single tissue, *i.e.* an antagonism as direct as that of heat and cold.

Antagonism between strychnine and veratrine (or any other poison decreasing muscular chronaxie, *e.g.* physostigmine, pilocarpine, etc.) is more complicated. Strychnine decreases nervous chronaxie, that is to say, it acts on the nerve in the same manner as the above-mentioned poisons on the muscle; hence, being able to curarize by producing heterochronism between the altered nerve and the normal muscle, it is able to bring nervous chronaxie close again to chronaxie of the poisoned muscle, and thus to re-establish functional connection by restoring isochronism. Such a statement is evidently too schematic for interpreting in their complexity all physiological events; for instance, alteration of the muscle is not a mere change of chronaxie; with the dose of veratrine required for curarizing, much stronger than the dose producing the well-known "veratrine twitch," contractility is conspicuously diminished; thus, contraction, when indirect stimulation reappears, cannot be as powerful as in the normal state. There are still some other obscure complications; nervous conductivity too seems to be diminished by strychnine. Nevertheless, indirect excitability springing up again after its entire loss appears as a striking fact when simply looking at the muscle.

But veratrine, physostigmine and every poison recognized as decreasing muscular chronaxie, are subject, with a strong dose and in a

secondary phase, to increase it sufficiently to curarize in this second manner, by a curare-like action *stricto sensu*. We were not aware of this particular when publishing our first experiments on veratrine [1912], but we discovered it with nicotine [M. Lapicque, 1922], and we generalized it some years later [L. Lapicque, 1930].

Of course, in their second phase, where curarizing by increase of muscular chronaxie, these poisons are no longer antagonized by strychnine, which always decreases nervous chronaxie. It happens that in one and the same experiment both kinds of curarization follow one another, allowing an opportunity for proving the efficiency of strychnine in the first one, and its lack of effect in the second one.

May 15, 1933. Gastrocnemius of *R. esculata* attached to a nearly isometric myograph; stimulation by nerve every half-minute during the whole experiment (Fig. 5). Before the

Fig. 5. Pilocarpine-strychnine antagonism. See text.

poison: indirect chronaxie 60, direct chronaxie 55. At point *P*, application of a solution of pilocarpine nitrate (0·1 p.c. in Ringer). The direct chronaxies are successively, at moments marked with crosses, 50–40–35. Contraction disappears; at this moment, chronaxie equals 30. Application of strychnine sulphate (0·2 p.c. in Ringer) especially on the motor point; after 1 or 2 min., recovery of the contractions which increase for about 3 min. maintain themselves for about 5 min. at a constant height nearly equal to the original height, and then slowly decrease. About ½ hour after the reappearance of the contractions a new and abundant instillation of pilocarpine, at point *P*, produces a slight and fugitive increase in height, followed by a rapid descent towards zero, *i.e.* a new curarization. An abundant instillation of strychnine is completely without effect. Direct chronaxie is 130.

In other cases it happens inversely that muscular chronaxie does not decrease enough in the first phase to attain curarizing heterochronism; consequently curarization can be realized only in the second manner, and thus, is not to be cured by strychnine. Such is generally the case in spring; we met with it when Rushton's last paper having appeared with negative results, we resumed at once with veratrine our former

experiments; we have often failed just as Rushton did. But by testing simultaneously indirect excitability, and both nervous and muscular

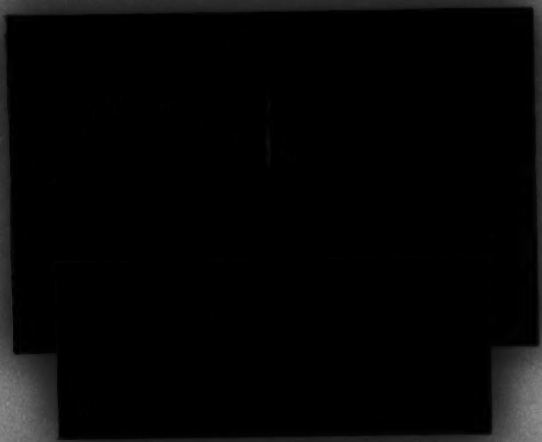

Fig. 6. Two experiments on veratrine-strychnine antagonism. At each cross veratrine solution is poured on the muscle. Curarization occurs. At *S*, strychnine solution is poured on; prompt recovery of indirect excitability.

Fig. 7. Veratrine-strychnine antagonism. Same experiment as in Fig. 6, but graph taken on the cylinder turning at a medium speed.

chronaxie, we came to a clear comprehension of this negative result. Nervous chronaxie appears in spring to be shorter than muscular chronaxie; on the other hand, muscle is liable under action of veratrine to

reach sooner than in autumn the phase of increased chronaxie. Thus it is difficult, in this season, to obtain curarization in the first phase; when it is obtained, it can be cured by strychnine; but if we perceive muscular chronaxie, after some decrease, beginning to increase again before indirect excitability has disappeared, we can be sure it is useless to try antagonism with strychnine.

That is to say, negative results as well as positive results are in accordance with the chronaxical theory.

We had succeeded, nevertheless, in the said season in registering the revival of the contraction under the influence of strychnine. This has been attained more easily on the toad than on the frog; veratrine contracture, as known, is particularly intense with this animal; so a complication is introduced in the graph, but it permits us all the same to see clearly the denied phenomenon.

Our experiments in 1912 succeeded without difficulty, as we then stated; they were made in autumn, as is printed in our original publication. During last autumn, the present paper being already in the editors' hands, we have, in numerous trials, obtained successful antagonism in nearly nine cases out of ten; nevertheless, we had the opportunity of observing some conditions of failure; namely, having killed a frog and taken one of its legs, and made a successful experiment with it, if we took the other leg, one or two hours later, it gave a negative result; this seems to depend on the state of asphyxia of this second leg, because curarization occurred only by increase of muscular chronaxie.

Rushton does not mention any measurement of chronaxie in his unsuccessful experiments; according to supplementary information, a good many of his experiments were performed in spring. On the other hand, asphyxiating conditions were probably present in his experiments. If Rushton had made the required tests, it is likely that he also would have observed an increased muscular chronaxie as soon as curarization appeared. Consequently, strychnine by decreasing nervous chronaxie, could not re-establish either isochronism or indirect excitability. Consequently also, Rushton's negative result agrees as well as our positive result with the chronological theory of curarization.

Summary.

1. Attention is drawn to the physiological proofs which show that muscular chronaxie can be measured only with stigmatic electrodes, and not at all with large fluid electrodes.

2. With convenient electrodes, chronaxie measurements on the aneural part of the sartorius, *i.e.* pure muscular substance, give values approximately equal to the known values for nervous chronaxie. Moreover, on the sartorius of *Bufo pantherinus*, it has been possible by simultaneous measurements on a muscle and on its own nerve to observe directly neuro-muscular isochronism.

3. The so-called Grundfest heterochronism, obtained by using capillary electrodes under Ringer, is shown to depend on an error of the measurement of nervous chronaxie, this being falsified, according to a known law, by too small a distance between the actual electrodes.

4. Even on the gastrocnemius, when stimulating electrically with stigmatic electrodes directly applied upon it, true muscular chronaxie can sometimes be obtained and clearly distinguished, owing to its evolution under the action of poisons.

5. A few of our old experiments on the curarization of the frog or toad gastrocnemius are given with unpublished details, showing that the disconnection is produced in the course of a continuous evolution of direct excitability, without observing any discontinuity in the curve.

6. Increase of chronaxie by curare is observed on the aneural part of sartorius.

7. Mineral substances, notably calcium, cannot be responsible for the above-mentioned increase of chronaxie. On the other hand, a pure organic substance with the same curarizing effect as curare, *e.g.* sparteine, does possess such an action.

8. On sartorius of *Bufo pantherinus*, on following evolution of pure muscular chronaxie under action of curare, it has been possible to observe accurately the point where indirect excitability disappears; disconnection between nerve and muscle is produced at the moment where the increased chronaxie, the evolution of which continues further, reaches a given value above the normal.

9. Rushton's so-called curarization experiments without change in muscular chronaxie are explained by a spontaneous alteration of the sartorius without any necessity for taking curare into account.

10. The same explanation should be applied to Rushton's negative test of my experiment with progressive currents.

11. New experimental evidence is given for antagonism between strychnine and veratrine or pilocarpine.

12. It is pointed out that poisons such as veratrine which decrease muscular chronaxie offer a second phase of action in which they increase chronaxie. Curarization may be produced in either one or the other

phase. Strychnine which always decreases nerve chronaxie, can evidently only in the first phase and not in the second, re-establish isochronism, and consequently indirect excitability. Rushton's negative experiments have very likely met with this second case.

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MODIFICATIONS IN THE USE OF THE GLASS ELECTRODE FOR THE DETERMINATION OF THE *p*H OF VENOUS BLOOD.

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(*Received November* 17, 1933.)

The method herein described was evolved with the object of determining the hydrogen ion concentration of whole, fresh, unadulterated blood at body temperature; *i.e.* under conditions approaching as nearly as possible those obtaining in the circulation. The glass electrode method was chosen since it is now recognized to be superior not only to colorimetric methods but to the other electrometric methods—at any rate for use with blood. But the difficulties of making *p*H determinations on whole fresh blood with the glass electrode have led workers in this field to use blood serum or whole blood to which extraneous chemical substances have been added to prevent coagulation and glycolysis which occur when unadulterated shed blood is allowed to stand. These difficulties with the use of whole fresh blood are mainly:

(1) Glycolysis, causing a progressive acid change in the blood.
(2) The difficulty of avoiding CO_2 loss.
(3) The difficulty of accurate temperature control.

In the case of whole blood, these difficulties have not been completely overcome without the addition of chemical substances such as potassium oxalate and sodium fluoride, which it is the object of the method about to be described to avoid. In particular the problem of accurate temperature control has not been satisfactorily solved. Various methods have been employed with the object of overcoming these difficulties, the chief of which are referred to below.

1. Glycolysis.

It has been shown by Lovatt Evans [1922] that the acid change which occurs soon after blood is shed is due to the conversion of glucose into lactic acid and can be prevented by the addition of sodium fluoride

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to the blood. Havard and Kerridge [1929] have determined the *p*H of whole blood without the addition of fluoride immediately the blood was withdrawn and before glycolysis had occurred.

The effect of glycolysis is not apparent in shed blood if kept at 0° C. until after the lapse of several hours. It occurs much more rapidly at a higher temperature. It has been shown by Peters, Barr and Rule [1921] that even at 38° C. the changes due to glycolysis are not observed until 20 min. after the withdrawal of the blood. They estimated the *p*H of whole blood on unadulterated specimens and on specimens to which sodium fluoride had been added, and found that in the former case the *p*H remained constant for 20 min. after the withdrawal of the blood, after which time an acid change set in. In the latter case they found that fluoride prevented the acid change altogether. In an experiment by Havard and Kerridge [1929] no change in the *p*H of the blood due to glycolysis occurred before the lapse of 14 min. from the withdrawal of the blood from the vein. In the method described here the *p*H is determined within 2 min. of the withdrawal of the blood, and therefore before any change due to glycolysis has occurred.

2. CO_2 loss.

This has been minimized by withdrawing blood from a vein into a syringe which contains paraffin in the blank space above the piston [Havard and Kerridge, 1929]. Actual contact of paraffin with the blood must be avoided, since the contact of paraffin with the glass membrane fouls the electrode. For this reason it is impossible to transfer the blood from the syringe to the glass electrode under paraffin, and even though it is stated that the blood may be subsequently covered with paraffin, the initial filling of the electrode by air displacement renders the blood liable to CO_2 loss. Buffer displacement is preferable but it involves a certain degree of dilution of the blood, and while this fact in itself may be of no great moment, it places the blood in the category of blood to which extraneous substances have been added.

In the present method CO_2 loss is eliminated completely by the manner of withdrawal of the blood and by the use of a Kerridge type of glass electrode specially modified for the purpose. This modification, of which an illustration is given, was made by Messrs Dixon and Co., 27 Devonshire Street, London, W.C. 1, and consists of the ordinary Kerridge electrode with a glass tubular prolongation upwards of the inner chamber (*A*) with a side tube (*B*) leading from it (see Fig. 1).

Blood is withdrawn from an antecubital vein, as far as possible without stasis, by means of a wide-bore needle to which is attached 6 in. of narrow rubber tubing. About 2 in. from the end of the tubing distal to the needle there is fitted a rubber cap which, when in position, accurately fits the opening in the electrode at *B* rendering it airtight. Before it is placed in position, however, blood is allowed to flow through the needle and tubing, so expressing any air that may be contained in the lumen. With the needle still in the vein the free end of the tubing is then inserted into the inner chamber of the glass electrode and the rubber cap is fitted

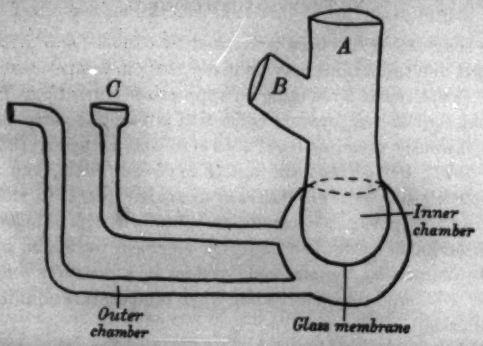

Fig. 1. Modified Kerridge type of glass electrode. The opening at *B* is closed by a tight-fitting rubber cap through which enters the rubber tubing. The open end of the tubing lies at the bottom of the inner chamber immediately over the glass membrane. After the electrode has been filled with blood it is fitted to the calomel electrode, one limb of which enters the inner chamber of the glass electrode through *A*, the opening of which is sealed off by a tight-fitting rubber stopper fitted to the limb of the calomel electrode. The two calomel electrodes dipping into the glass electrode at the points *A* and *C* are connected to a slide wire potentiometer and an electrometer valve amplifier.

closely over opening *B* (see Fig. 1). In this position the open end of the tubing lies at the extreme bottom of the inner chamber immediately over the glass membrane and the inner chamber fills up with blood. The lower end of the tubing is covered when only 1 or 2 c.c. of blood has entered the glass electrode, so that only the first 1 or 2 c.c. of blood that enters the electrode vessel comes into contact with air. The electrode vessel fills up to the top of *A* (Fig. 1) and overflows. Meanwhile the electrode vessel continues to fill from the bottom and the blood now entering it at no time comes into contact with air, even momentarily. The small quantity

of air-exposed blood has risen to the top (having been displaced upwards) and has overflowed, and is not used. A clip is now placed on the rubber tubing, the needle is removed from the vein and the glass electrode fitted to the calomel electrode. One limb of the calomel electrode enters the inner vessel of the glass electrode through *A*, the opening of which is sealed off by a tight-fitting rubber stopper which is fixed to the limb of the calomel electrode. A reading is taken immediately and the *p*H so determined within 2 min. of the withdrawal of the blood from the vein.

3. Temperature control.

When coagulation has been prevented by oxalate, and glycolysis by fluoride, *p*H determinations may be made at room temperature, and the *p*H at 38° C. arrived at by making an appropriate correction. The objection to making this temperature correction is that the correction factor has been variously given as 0·011 [Martin and Lepper, 1926], 0·012 [Cullen, 1922], 0·015 [Havard and Kerridge, 1929], and by other observers 0·018 and 0·02 *p*H decrease in acidity per 1° C. rise in temperature, and very variable results are obtained depending on which correction factor is used. The difficulty has been overcome by several workers by immersing the electrode system in a constant temperature air bath at 38° C. and taking a reading when temperature equilibrium has been established.

When, however, fresh blood is used, to which neither oxalate nor fluoride has been added, it is felt that the difficulty of temperature control cannot be satisfactorily overcome by this method. Havard and Kerridge [1929], employing unadulterated whole blood, have used the method of withdrawing blood (at room temperature) into a syringe which has been previously warmed to 38° C. (or in certain circumstances to 45° C.) and immediately transferring the blood to the glass electrode which is immersed in a constant temperature air bath at 38° C. A reading is taken almost immediately before glycolysis could have occurred. Although accurate results are claimed it is felt that a true reading could not be obtained immediately by this method since temperature equilibrium between blood at an uncertain temperature and buffer solution at only an approximately equal temperature (38° C.) cannot be obtained until after the lapse of some time. In this method it would be impracticable to wait for the establishment of temperature equilibrium between blood and buffer owing to the risk of CO_2 loss and possibly the onset of acid changes due to glycolysis.

The immersion of blood which has been treated with oxalate and fluoride in a constant temperature air bath at 38° C. is a satisfactory method of temperature control since ample time may be allowed for temperature equilibrium to become established. But the method is unreliable with fresh unadulterated blood (unless an extremely small quantity is used) since glycolysis or CO_2 loss may occur while temperature equilibrium is being established—the time required for which is an uncertain and variable factor. For this reason the method was ultimately adopted in this investigation of making all *p*H determinations in a room heated to body temperature. Platt and Dickinson [1933] have recently described a similar method of temperature control, which however requires much more elaborate apparatus. Apart from the discomfort of working in a room at 98° F., the method has obvious advantages over those previously employed with the use of fresh blood to which the addition of chemical substances has been avoided. It is possible that the *p*H under these conditions is slightly on the alkaline side when compared with the *p*H taken at normal room temperature. For purposes of comparison however this is irrelevant since all *p*H determinations are carried out under constant conditions. In any case any such change would be so small as to be negligible.

The room is heated to 38° C. and maintained at that temperature for 1½ hours before use. At the end of this time the whole electrode system has attained blood temperature. The subject then enters the room and remains seated for 5 min. before blood is withdrawn into the glass electrode. A reading is taken immediately, *i.e.* within 2 min. of the blood leaving the vein, but not before equilibrium has been established. (Stadie, O'Brien and Lang [1931] have shown that it requires only 30 sec. for blood to attain equilibrium in a glass electrode.)

It is believed that this method of temperature control and of avoidance of CO_2 loss is an advance on methods hitherto used in that the addition of oxalate and fluoride to the blood has been avoided. It is the only method which in our hands has yielded reliable results in a large series of cases. It should be mentioned that the results obtained from normal subjects come within the range of generally accepted normal values.

In our cases, under normal resting conditions, the lowest *p*H recorded was 7·27 and the highest 7·43, with an average of *p*H 7·36. It may be mentioned that in none of our cases was an acid change observed which corresponded to that described by Havard and Kerridge [1929]. This is in agreement with the finding of Platt and Dickinson [1933] who believe that this so-called acid change was due to faulty temperature control.

SUMMARY.

1. The difficulties encountered in the accurate determination of the *p*H of whole blood without the addition of oxalate and fluoride are discussed.

2. A technique to overcome these difficulties is described together with a suitable modification of the Kerridge type of glass electrode.

We should like to express our gratitude to Prof. de Wesselow for looking over the paper.

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612.173:615.361.37

THE ACTION OF INSULIN ON THE R.Q., OXYGEN UTILIZATION, CO_2 PRODUCTION AND SUGAR UTILIZATION IN THE MAMMALIAN DIABETIC HEART.

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(*Received December* 4, 1933.)

The purpose of this investigation was to present a record of the changes of the gaseous metabolism of the diabetic heart as they were effected by insulin and to attempt to determine whether, as far as cardiac muscle is concerned, the essential feature of diabetes is a failure of the cardiac tissue to oxidize carbohydrate or an over-production of carbohydrate from fat. The hypothesis that oxidation is primarily at fault in diabetes is coupled with the belief that fatty acids cannot be converted into glucose. Although Lusk may years ago maintained that the idea of a transformation of fat into sugar was a superstition there are still those who believe otherwise. While the results to be discussed here form a contribution to this controversy it must be remembered that they are obtained from isolated hearts, and the extent to which they may be accepted as evidence for or against will depend upon how far one is prepared to go in accepting and applying to the whole animal results obtained from an isolated organ.

Methods.

Amytal was used as the anæsthetic. Blood sugar was estimated by the (No. 50) modification of the Shaffer-Hartman [1920] method, described by Shaffer and Somogyi [1933], muscle glycogen by the modification described by Sahyun [1931] and the gaseous metabolism by the method previously described [Cruickshank and Startup, 1933*a*]. All the experiments were carried out on isolated heart-lung preparations from dogs not less than 7 days diabetic. To the circulating

blood which amounted to about 500 c.c., 0·5–1 c.c. of 1 : 100,000 adrenaline in saline was given every half-hour; the blood-pressure was thus maintained at or above 100 mm. Hg, an active heart ensured and the onset of œdema of the lungs postponed for at least 2 hours.

Results.

That the R.Q. of the whole diabetic animal is of the order of 0·71 is seen from Table I. The injection of 10 units of insulin to the animal raises the R.Q. towards unity within 30 min.; it is to be noted that insulin produces but little increase in oxygen consumption, accompanying a definite rise in CO_2 production, and also that its effect passes off almost completely within 1 hour.

Table I. Showing effect of insulin on the R.Q. of an animal 7 days diabetic.

Period 30 min.	CO_2 c.c.	O_2	R.Q.	Remarks
Exp. 9. iii. 33. Wt. of dog = 6·5 kg.				
1	1282·7	1748·0	0·73	No insulin
2	1247·1	1895·2	0·66	No insulin
Exp. 4. iv. 33. Wt. of dog = 4·3 kg.				
1	619·2	823·8	0·75	No insulin
2	763·5	844·5	0·91	10 units
3	682·1	873·5	0·78	No insulin
4	587·1	814·9	0·72	No insulin
5	788·8	811·4	0·98	10 units
6	717·3	796·0	0·90	10 units

In Table II are set down the details of the experimental results on the isolated heart. In all cases the first period is a record of events 1 hour after the commencement of isolation of the heart. The first half-hour period of such experiments often shows an excessive production of CO_2; it is therefore essential to wait for a steady state in the gaseous exchange and in cardiac action.

The R.Q. oxygen consumption and CO_2 production.

In the markedly diabetic animal the R.Q. is 0·70. That the R.Q. of the mammalian heart falls after pancreatectomy was first demonstrated by Starling and Evans [1914–15], but although their results showed great variations they stated that the R.Q. of the diabetic heart has an average value of 0·71, and concluded therefrom that the muscle was unable to utilize carbohydrate. They found that the addition of normal blood to the diabetic heart was without any marked effect, but in one experiment a change in R.Q. from 0·73 to 0·79 is recorded. They reported a pro-

TABLE II. Showing the effect of insulin on the R.Q., CO_2 production, oxygen utilization, sugar oxidation and glycogen synthesis in the diabetic heart.

I Period 30 min.	II CO_2 c.c./g./hr.	III O_2 c.c./g./hr.	IV R.Q.	V Blood volume c.c.	VI Blood sugar g./100 c.c.	VII Blood sugar removed mg./g./hr.	VIII Sugar oxidized mg./g./hr.	IX Sugar stored mg./g./hr.	X Ratio VIII/IX	XI Cal./g./hr.	XII Insulin
Exp. I. Weight of heart = 50 g. Glycogen = 1·49 p.c.											
1	3·46	4·84	0·71	530	0·384	0·63	0·08	0·55	0·15	22·70	None
2	4·27	5·12	0·83	530	0·381	4·13	2·80	1·33	2·10	24·76	5 units
3	4·88	4·86	1·00	520	0·362	8·01	6·48	1·53	4·20	24·52	5 units
					0·323						
Exp. II. Weight of heart = 47 g. Glycogen = 0·97 p.c.											
1	3·88	5·50	0·70	600	0·206	2·55	—	2·55	—	25·80	None
2	4·66	5·54	0·84	600	0·216	5·90	3·32	2·58	1·3	26·87	5 units
3	5·11	5·50	0·93	590	0·193	8·70	5·57	3·13	1·8	27·28	5 units
4	5·16	5·26	0·98	570	0·158	7·28	6·52	0·76	8·6	26·41	5 units
					0·128						
Exp. III. Weight of heart = 70 g. Glycogen = 1·45 p.c.											
1	3·78	5·20	0·72	475	0·310	2·71	0·21	2·50	0·08	24·44	None
2	5·23	5·32	0·98	470	0·290	7·52	6·60	0·98	6·80	26·71	5 units
3	4·22	5·37	0·77	470	0·234	2·69	1·55	1·14	1·37	25·57	None
					0·214						

Note. The sugar oxidized is calculated from the oxygen used and the R.Q., the difference between this and the sugar removed from the blood gives the carbohydrate stored.

nounced rise on the addition of a neutralized HCl extract of pancreas. We find that the addition of insulin to the blood perfusing the heart raises the R.Q. from that indicating fat metabolism, 0·70, to unity: this is due to an increase in CO_2 production. In the first and third experiments (Table II) when the R.Q. rose from 0·71 to 1·07 and from 0·72 to 0·98 the CO_2 production rose 41·0 and 38·4 p.c. respectively; in the second experiment the R.Q. rose from 0·84 to 0·98, the CO_2 production increased 10·5 p.c. The change in oxygen consumption was in each case almost negligible, approximately 5 p.c. (Fig. 1 and Table II). For the diabetic heart the average figure for oxygen consumption is, at R.Q. = 0·71, 5·00 c.c. per gram of heart muscle per hour; for the normal heart it is 4·00 c.c./g./hour; with the increase in the R.Q. to unity due to the addition of insulin it rises but slightly to 5·14 c.c./g./hour.

Changes in CO_2 production are however of an entirely different order; from a diabetic average (R.Q. = 0·7) of 3·62 c.c./g./hour, it is raised to 5·09 c.c. with an R.Q. of unity, or 40·6 p.c. for the complete change from fat to carbohydrate metabolism as effected by insulin.

The energy expenditure of the heart has been calculated from the calorific value of a litre of oxygen for the R.Q. obtaining, or it may be calculated from the amount of CO_2 produced, determining from the R.Q. the amount of fat and/or carbohydrate burned. Such calculations show that the change in metabolism is virtually equicaloric, a fact previously noted as an effect of insulin by Cori and Cori [1928].

Sugar oxidation and glycogen synthesis.

The amount of sugar which disappears from the blood depends upon the degree of diabetes. The isolated heart of a dog, 5 days diabetic, may show a sugar utilization of 1·15 mg./g./hour, while a preparation 9 days diabetic may show only 0·09 mg./g./hour [Cruickshank and Shrivastava, 1930]. With insulin such low figures as are shown in Table II and in Fig. 1 are raised well beyond the normal, from practically zero at the R.Q. 0·71, to 6·52 mg./g./hour at the R.Q. unity, which would indicate complete restoration of the power of oxidation of carbohydrate by heart muscle.

Of interest is the ratio, oxidation/synthesis, in the heart treated with insulin. While with a low R.Q. the amount of blood sugar stored as glycogen is large in comparison with that oxidized, the absolute figures are extremely small. This is doubtless due to the fact that the diabetic heart contains already a definitely greater percentage of glycogen than does the normal [Cruickshank, 1913]. In a series of five experiments

the hearts of bled dogs, 1 week diabetic, contained an average of 1·25 g./100 g. (maximum 1·71, minimum 0·99), while hearts after 2 hours' perfusion with blood to which insulin had been added contained 1·18 g./100 g. (maximum 1·50, minimum 0·97). The average glycogen content of the normal heart may be taken as 0·60 g./100 g. [Cruickshank and Startup, 1933*b*].

These average figures are much higher than those previously obtained, namely 0·48 and 0·71 g./100 g. for the normal and diabetic heart, and

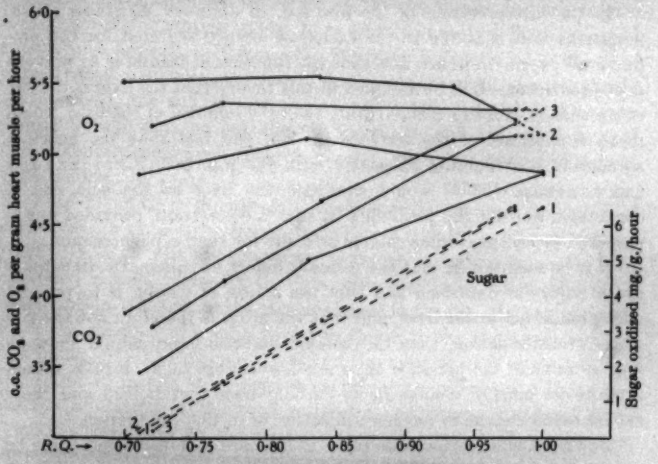

Fig. 1. Showing the relation of CO_2 production, O_2 utilisation and sugar oxidized per gram of heart muscle per hour, to the changes in the R.Q.

the estimations show a far greater consistency than those of earlier workers, due in all probability to improvements in the original Pflüger method [Sahyun, 1931]. Such high figures for the glycogen of diabetic hearts would indicate, as was suggested by Knowlton and Starling [1912] and Maclean and Smedley [1913] that the heart had lost completely the power of utilizing its carbohydrate stores, but not the power of synthesis.

Columns VII, VIII and IX of Table II show the relation between the oxidation of sugar and glycogen synthesis in the diabetic heart with and without insulin; oxidation is markedly enhanced while synthesis is

reduced to a minimum. This latter effect does not annul the fact that in normal hearts the essential activity of insulin is to promote glycogen synthesis, for as has just been shown the diabetic heart is surcharged with glycogen and further storage of carbohydrate is probably impossible. It, however, allows of a very clear demonstration of what is apparently the chief function of insulin in the diabetic heart, namely an increase in the oxidation of sugar.

Discussion.

In the interpretation of the low R.Q. in diabetes Macleod [1928] maintains that it is due to the excess of oxygen required for the production of sugar from fat, and that the function of insulin is to prevent over-production. If it be assumed in this theory that the liver is the site of conversion of fat to carbohydrate then the removal of the liver would mean a cessation of the conversion. But the fact that the heart in aglycæmia can function efficiently with the R.Q. 0·70 [Cruickshank and Startup, 1933*b*] would eliminate the liver as the sole site of conversion but not the possibility of such a conversion, provided one is prepared to accept cardiac muscle as a site for such a phenomenon.

If it be maintained that the primary defect in pancreatic diabetes is not a failure of oxidation and that the action of insulin is to prevent over-production in the liver, and that the heart is therefore not the site of the transformation, then the isolated diabetic heart should show an R.Q. of unity in the presence of so much available sugar, insulin should produce no marked change in its carbohydrate metabolism and there should be no change in oxygen utilization or in CO_2 production.

But if transformation take place in the cardiac muscle and there be no impairment of oxidation, that is to postulate that in the diabetic heart carbohydrate is steadily being formed from fat, then the addition of insulin should diminish the production of carbohydrate and reduce the oxygen intake, but should not reduce the CO_2 output; the R.Q. should approach unity and that from an R.Q. previously not above 0·70. If this were so the rate of sugar disappearance from the circulating blood, that is the difference between sugar produced and sugar burned, should increase from a comparatively low figure to that of the normal sugar utilization.

From the results shown, this is exactly what happens with regard to the R.Q., which rises from about 0·72 to unity, and to the sugar utilization, which increases from zero to 6·50 mg./g./hour, but not with regard to the oxygen utilization in which there is no change, or the CO_2 production,

which has definitely increased, the increase for the change in R.Q. from 0·71 to unity being practically 40 p.c. The increase in CO_2 production coupled with the lack of change in oxygen consumption lends no support to the hypothesis that insulin controls the neoglucogenesis from fat in cardiac muscle. The assumption that the transformation of fat to sugar may take place in the heart muscle therefore receives no support from the facts cited.

If, however, we assume that carbohydrate oxidation is impaired in the diabetic heart and that no transformation of fat to sugar takes place in such a heart, then we are forced to the conclusion that the cardiac muscle is utilizing protein and/or fat as sources of energy. The R.Q. should therefore be between 0·70 and 0·81. If insulin restores the lost power of carbohydrate oxidation, the rate of disappearance of blood sugar should be increased, the R.Q. should rise to unity, the utilization of oxygen should be constant or but slightly increased, and the production of CO_2 definitely increased. This is exactly what has happened in our experiments.

That the R.Q. is increased in diabetic subjects upon injection of insulin has been clearly demonstrated by Fitz, Murphy and Grant [1922]. Krogh [1923] has shown that the injection of insulin into curarized rabbits increased the R.Q. with practically no change in O_2 consumption. That there is a similar change in the R.Q. of diabetic hearts upon the addition of insulin is clearly shown in the results set forth in this paper. Dale [1923] and Cori and Cori [1926] have suggested that the action of insulin is to increase carbohydrate oxidation without any definite alteration in the total respiratory exchange. Cori and Cori [1928] in their work on the disappearance of carbohydrate in the post-absorptive state in rats have shown that a change in the R.Q. from a normal of 0·82–0·94 by the injection of insulin is accompanied by a slight fall in O_2 used and an increase of 14·8 p.c. in the CO_2 produced, and they have shown further that there is an equicaloric replacement of carbohydrate oxidation for fat oxidation. This is in agreement with the results which we have obtained on the diabetic heart which show that any change from fat to carbohydrate metabolism does not entail any marked changes in calorific output.

That the rapid shifting of metabolism from fat to carbohydrate is a result of insulin action has been suggested by many [Dale, 1923; Krogh, 1923; Minkowski, 1924; Macleod, 1924, 1926]. The evidence for such a shift in the diabetic animal is not so vast, and is somewhat conflicting.

That there is a definite shift from fat to carbohydrate metabolism upon the addition of insulin to the blood perfusing the diabetic heart is evident. While the argument set forth upon these results does not rule out of consideration the theory of the production of sugar from fat, yet, in conjunction with the findings on the metabolism of the heart muscle in aglycæmia, they would certainly tend to weigh the balance of experimental evidence in favour of the theory that a failure in the oxidation of carbohydrate is the primary defect in pancreatic diabetes.

Summary.

The gaseous metabolism, sugar oxidation, oxygen utilization and CO_2 production have been investigated in the diabetic heart with and without insulin. In animals 7 or more days diabetic the R.Q. is 0·70 both for the whole animal and the isolated heart; the oxygen utilization in the diabetic heart is 5·00 c.c./g./hour, the CO_2 production is 3·62 c.c./g./hour; sugar oxidation is virtually absent.

The effect of insulin on the diabetic heart has been investigated. Upon the addition of insulin, 5 units per half-hour, the R.Q. rises from 0·70 to unity, the oxygen utilization remains steady, the CO_2 production increases 40 p.c., while sugar oxidized is raised to 6·52 mg./g./hour.

From these results it is concluded that the transformation of fat to sugar does not take place in cardiac muscle and that the primary defect in the diabetic heart is a failure to oxidize carbohydrate.

The average glycogen content of the diabetic heart has been found to be 1·18 g./100 g. Insulin has little power to increase glycogen synthesis in a heart so highly charged with reserve carbohydrate.

We wish to express our thanks to Messrs Eli Lilly and Co. for the generous manner in which they have continued to supply us with iletin and amytal.

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545.83:537.362

A MICRO METHOD FOR THE DETERMINATION OF BASE BY ELECTRODIALYSIS.

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(*Received January* 17, 1934.)

An electrolytic method for the determination of sodium and potassium in biological fluids has been described by Stoddard [1927]. The solution to be analysed is electrolysed in an apparatus consisting of two concentric tubes, communicating by means of apertures through the wall of the inner tube. Each tube contains mercury, that in the inner tube acting as the cathode. On electrolysis, positive ions in the solution migrate to the inner tube, where they form an amalgam, which is removed and titrated. The results obtained are consistent within 2 p.c., for samples containing base equivalent to from 2·5 to 17 mg. of sodium.

The present communication records a modification of the electrolytic method, adapted for analysis of quantities of total base equivalent to 0·2 mg. of sodium. In working with very small amounts of base it is desirable to eliminate the preliminary processes for the removal of protein and phosphates, described by Stoddard, and for this purpose we have used a mercury cathode separated by a collodion diaphragm from the solution containing base. On electrolysis, positive ions migrate through the collodion membrane and form an amalgam with the mercury, which reacts with a measured amount of standard 0·01 *N* sulphuric acid placed above it. After electrolysis, the excess of acid is determined by titration with 0·10 *N* sodium hydroxide, using a Rehberg microburette [Rehberg, 1925]. Syringe pipettes [Krogh and Keys, 1931] may be used to obtain an accuracy greater than 0·02 p.c. in the delivery of the acid. A mercury anode is used, so that in the case of solutions containing chlorides, no free chlorine is liberated. The formation of insoluble mercury salts at the anode causes a great increase in the resistance of the solution, and the diminution of the current affords an index of the progress of the reaction.

Apparatus.

For electrolysis we have used a source of direct current of 100–250 volts, and a variable resistance, which may be required to prevent overheating during the first few minutes of electrodialysis. The circuit was so arranged that three experiments could be carried out simultaneously.

The anode vessel is a tapered glass tube of 7·5 cm. length and 2 cm. diameter, with a short platinum wire sealed into the tapered end. The tube contains 0·3 c.c. of mercury. The anode vessel is placed in a 20 c.c. beaker containing dilute sulphuric acid, in which a platinum wire electrode is immersed. The rate at which bubbles are produced in the sulphuric acid affords an indication of the progress and conclusion of dialysis. The cathode vessel is prepared by fixing a collodion tube of about 4 cm. length on to a piece of glass tubing of 9·5 cm. length and 1·5 cm. diameter. The glass tubing is indented at one end, so that it can be supported conveniently in the rack of the Rehberg burette (Fig. 1).

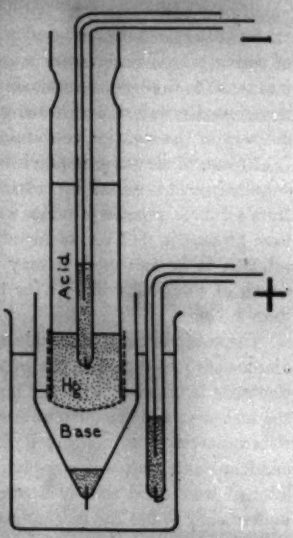

Fig. 1. Apparatus for electrodialysis. The collodion membrane is represented by the broken line.

The collodion membranes have been prepared from one volume of "Necol" dissolved in one volume of absolute alcohol and one volume of anhydrous ether. A test-tube of 1·5 cm. diameter, with a small, even hole in the centre of its base, is fixed on a rod which is rotated mechanically; an electric heater is arranged so that the temperature in the region of the rod is about 40°. Three coats of collodion are poured over the glass tube, an interval of 2 min. being allowed for drying between the application of each coat; finally, the membrane is left to dry for 5 min. The test-tube is then put into water and the membrane can be removed a few minutes later. After cutting it to the necessary length, the top of the membrane is softened with alcohol and ether, and it is drawn over the cathode tube so that the base of the membrane is stretched tightly over the end of the tube. A few drops of

water are put inside, and the tube is placed in front of the electric heater with the collodion diaphragm just dipping into water, until the collodion in contact with glass has dried, when it should grip the sides of the glass tube firmly at all points. It is essential that the diaphragm should be kept in water, as drying causes it to become impermeable.

Experimental procedure.

The solution to be analysed is placed in the anode vessel over the layer of mercury, and if necessary a known volume of water is added, so that it is possible to place the cathode at a distance above the anode sufficient to reduce the risk of overheating. Syringe pipettes may be used for the delivery of the solution containing base.

1·5 c.c. of mercury is put into the cathode vessel and standard 0·01 sulphuric acid is added by means of a syringe pipette. A single delivery from a 1·5 c.c. pipette provides a sufficient excess of acid to react with the base present in 0·05 c.c. of blood serum. If ammonia be present in the solution to be analysed, it may be advisable to add water so that the depth of solution available for the absorbtion of ammonia be not less than 2·5 cm.

The cathode vessel is fixed in a small retort stand, so that the collodion diaphragm just dips into the solution containing base. A platinum wire electrode is inserted, with the point at least 3 mm. below the surface of the mercury, and the current is switched on. In our earlier experiments electrodialysis was continued for 15 min. and the reaction between amalgam and acid was completed by the use of a mechanical shaker; later, it was found that if electrodialysis be continued for 30 min., it is unnecessary to use the mechanical shaker. At the end of this period the cathode platinum electrode is raised and washed down with 1 c.c. of water; the cathode vessel is gently shaken, avoiding contact of the acid solution with the collodion diaphragm, the excess acid is then titrated with 0·1 *N* soda, a small measured quantity of methyl red being added as indicator. Titration is carried out as described by Rehberg [1925]. After titration the cathode vessel is immediately washed out and put into water, to prevent drying of the collodion.

It is essential to use purified mercury for anode and cathode, and glass distilled water for the preparation of solutions and all subsequent operations. Each sample of mercury and of distilled water should be tested by blank determinations and subjected to further purification if the blank exceeds 2 c.mm. of 0·1 *N* soda, equivalent to 0·0046 mg. sodium. New membranes, and membranes which have been stored for

some time must be tested by blank determinations and by measurements on known solutions, to ensure that no base is occluded in the membrane; if high results be obtained, the process of electrodialysis should be repeated until impurities are removed.

The permeability of the membranes may be tested by experiments on known solutions; if the rate of electrolysis be slow, and the base added be not completely recovered in the time allotted for dialysis, the membrane must be rejected. Membranes of low permeability are unsuitable for analysis of solutions containing protein, as when electrolysis is slow, a coherent layer of coagulated protein may be formed over the surface of the membrane. Stirring may be necessary for solutions containing much protein.

Results obtained by the application of the method to known solutions are recorded in Table I. The quantities of base taken in each experiment

TABLE I.

Known solution in milli-equivalents per litre	Observed values
15·0 ammonium phosphate	14·88, 14·91, 14·87, 14·98, 14·83, 14·89, 14·92, 14·94
15·0 ammonium phosphate with 0·1 c.c. 19 p.c. hæmoglobin	15·01, 15·13, 14·98, 15·16, 14·96
15·0 ammonium phosphate with 1·0 c.c. 19 p.c. hæmoglobin	14·19, 14·98, 14·71, 14·89
15·0 sodium chloride	14·80, 14·87, 14·92, 15·00
20·0 sodium acetate	20·07, 20·23, 19·30, 20·11
15·0 sodium chloride with 0·1 c.c. 19 p.c. hæmoglobin	14·77, 14·87, 14·82
10·0 potassium phosphate	10·07, 9·98, 9·81
Normal horse serum (0·06 c.c. per determination)	148·3, 152·0, 150·8, 147·3, 149·0

ranged from 0·009 to 0·024 milli-equivalents. The percentage accuracy is not inferior to that given by Stoddard's method. In his work analyses on known solutions showed that 1·2 p.c. of the total potassium and sodium is not recovered, and a correction is introduced to allow for this loss. In the present investigation it has not been found possible to give a correction factor applicable to all solutions. In the case of a number of solutions containing univalent ions, recorded in Table I, such a factor seems unnecessary. In a series of experiments on calcium chloride, not recorded in the table, approximately 95 p.c. of the total base was recovered. Theoretically, there are a number of factors which may prevent a complete recovery of the base, such as the formation during electrolysis of insoluble precipitates containing base, and the

presence of complex ions which are not dissociated under the conditions of the experiment. In view of the possibility that the percentage recovery of base may depend upon the composition of the solution, before application of the method, it is advisable to carry out experiments on solutions of similar composition containing known amounts of base, in order to determine any necessary correction factors.

SUMMARY.

A micro method for the determination of total base by electrodialysis is described. The method has been applied to certain mixtures of proteins and univalent bases, including ammonium, sodium, and potassium.

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612.386.084.1

A METHOD FOR DETERMINING IN ANIMALS THE ALIMENTARY ABSORPTION TIME FOR WATER, THE ABDOMEN REMAINING INTACT.

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(*Received January* 31, 1934.)

It is sometimes desirable to know the rate at which a dose of water administered by mouth is absorbed from the alimentary tract of a laboratory animal. In man the absorption time may be determined by observing the fall in the "weight" of the abdomen which takes place during the absorption of the water [Smirk, 1933]. The following experiments, which uphold the principle of the method already used in man, also show that the method is applicable to lower animals.

Experimental procedure.

The experiments were performed on cats decerebrated at the level of the tentorium. The apparatus used consists of a pharmacist's balance clamped to a rigid support *A*. (See Fig. 1.) Rigidity is essential. From one beam of the balance the scale pan and weights are suspended, from the other beam hangs a wire 100 cm. long. At the lower end of this wire is attached a sling *B*. The sling is best made of metal or wood, for it must not stretch or measurably change its shape in the course of the experiment. A light pointer is attached to the right-hand beam of the balance, and the scale *C*, graduated in millimetres, is placed behind the pointer so that slight deflections of the pointer are apparent.

The position of the animal is indicated on the figure. The anterior and posterior thirds rest upon the top of the box *D*, while from the xiphisternum to the symphysis pubis it is supported over a gap in the box *D* by means of the sling *B*.

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The animal, which lies on its back, is held in position by tapes which should not constrict the limbs, and their arrangement is shown in the figure. In the case of decerebrate animals which show any tendency to arching of the back it may be found better to bring the forelimbs to the side of the animal fixing the tapes at *F* instead of at *E*.

In order to determine the absorption time, a dose of water (say 80 c.c. for a 4 kg. cat) is given by means of a stomach tube, or the animal is

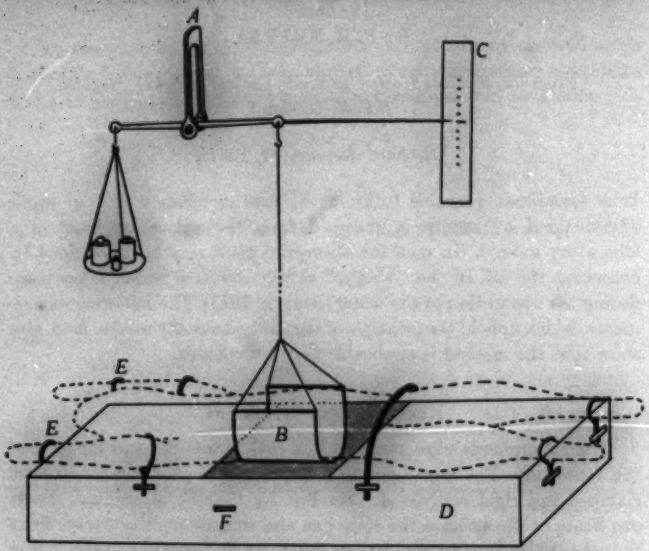

Fig. 1. Method for determining in animals the alimentary absorption time for water.

allowed to swallow it reflexly. The weights in the scale pan are then adjusted until the beam of the balance is horizontal and at the same time the body of the animal should be approximately horizontal. It will be found that the pointer registers movements of small amplitude in unison with respiration, and its average position should be noted accurately on the scale *C*. The time after giving the water when this observation was made should be recorded, and from this time until the conclusion of the experiment the pointer should be observed continuously. It is usually

found that within a few minutes the average position of the tip of the pointer has changed, it being ½ or 1 mm. higher on the scale *C* and a weight of 5 or 10 g. must be removed from the scale pan in order to restore it to its original range. Weights are removed from the scale pan as frequently as is necessary to keep the end of the pointer oscillating within ½ mm. of its initial range of movement. If the animal moves, the weights which have to be added or subtracted from the scale to correct the displacement are not counted.

It is very important that the water given should be at 37° C., for the method does not distinguish between vasomotor changes (of thermal origin) which alter the amount of blood in the abdomen and the changes in the abdominal weight due to the absorption of water.

Results.

In an animal without water which has been lying, and continues to lie, quietly and breathes regularly the weight required to counterbalance the abdomen remains almost constant. Any changes which take place are

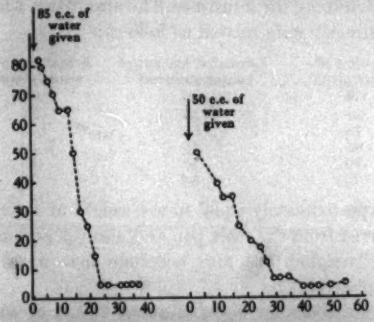

Fig. 2. The fall in the "weight" of the abdomen during water absorption (two successive doses of water given). Abscissæ: Time in minutes after giving water. Ordinate: Additional weight (g.) in the scale pan required to counterbalance the abdomen owing to previous water administration.

small and may be either in the direction of increase or decrease. If a weight of 20 or 30 g. is placed gently upon the abdomen a corresponding weight on the scale pan will balance it. Provided no reflex movements are induced, the change in the position of the pointer caused by the introduction of moderate doses of water into the stomach (or by their removal from the

stomach) is counterbalanced by corresponding increase (or decrease) of the weight on the scale pan.

If large doses of water are given (90 c.c. or more to a 4 kg. cat) the correspondence between the amount of water given and the counterpoise placed in the scale pan is less accurate. Such discrepancy (15–25 g.) is probably to be explained by displacement of the liver and by alterations in the weight of blood contained in the abdominal blood vessels.

The gradual decrease in the weight of the abdomen is illustrated by Fig. 2. It will be seen that when the "weight" of the abdomen has become steady the total loss in "weight" is approximately equal to the quantity of water given.

Several animals in the present series retained almost all the water in the stomach. In all such instances delay in or absence of absorption was first deduced by the indirect method described above and subsequently was verified by autopsy or by recovery of the unabsorbed water from the stomach by means of a rubber tube.

At various stages of absorption five other animals were killed and the amounts of water which had been absorbed were determined by emptying the stomach and milking the intestines. The absorption times determined directly and indirectly were related as follows:

Amount of water given c.c.	Estimated amount of water absorbed c.c.	Actual amount of water absorbed c.c.
80	35	39
75	65	53
95	35	20
60	40	35
75	All	70

When weights approximately equal to the weight of water administered have been removed from the scale pan and also the abdomen has ceased to diminish in "weight" one may conclude that water absorption is practically complete.

The absorption times for doses of about 70 c.c. of water in 10 cats weighing about 4 kg. were usually about 35 min.; occasionally as long as 40 min. by this indirect method. This may be compared with the observations of Klisiecki, Pickford, Rothschild and Verney [1933] who found that about 250 c.c. of water are absorbed by 10 kg. dogs in 36 min. and those of Heller and Smirk [1932] who found that the time expended in the absorption of 5 p.c. body weight of water averaged 40 min. in rats. In normal man the time taken to absorb 1 litre of water, as estimated by a method similar to the present one, was 22–55 min. [Smirk, 1933].

SUMMARY.

1. A method is described for animals by which the alimentary absorption time for water may be determined without opening the abdomen.

2. In normal activity decerebrate cats absorbed about 4 p.c. of their body weight of warm water in 35 min.

3. These observations on cats confirm the view that in man the changes in the abdominal "weight" which have been observed during the hour following water ingestion are due to and are a measure of water absorption.

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612.492:612.464.1

THE PITUITARY GLAND IN RELATION TO POLYURIA AND TO WATER DIURESIS.

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(*Received January* 31, 1934.)

The experiments described in this paper deal with the relationship between the pituitary gland and water diuresis. In 1913 Von den Velden discovered that the polyuria of diabetes insipidus was curtailed by injections of pituitary gland extract. Soon after Motzfeldt [1917] suggested that the pituitary gland regulates the excretion of water by the kidneys.

Animal preparations lacking the anatomical pituitary gland were then used by many workers to test this hypothesis; but the discovery subsequently of a strip of pituitary gland tissue, distinct from the anatomical gland and lying superficially in the hypothalamus, revealed serious difficulties in the interpretation of such experiments.

Starling and Verney [1925] approached the problem synthetically, perfusing their primary unit, the kidney, by means of a heart-lung preparation. In the course of perfusion their isolated kidneys developed a marked polyuria, the urine formed being dilute and especially poor, percentually, in chlorides. The urine flow and composition were restored to within normal limits by the addition of pituitary hormone to the perfusing blood. They suggested that substances having an action similar to that of pituitary hormone normally regulate the output of water and chloride in the intact animal. The absence from the blood stream of such substances was thought to be the essential cause for the diuresis after drinking water, for the ingravescent polyuria of isolated perfused kidneys of the dog, and for the polyuria of diabetes insipidus. The process of synthesis was extended by Verney [1926] who introduced into the

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perfusion system dog heads which contained the pituitary gland and heads from which the anatomical pituitary gland had been removed. Evidence was obtained that in this preparation the pituitary gland contributed an antidiuretic substance to the perfusate which prevented the inherent polyuria of the isolated perfused kidney. On this and other evidence Verney [1929] suggested that the polyuria of the isolated kidney was of the same nature as water diuresis. While giving full weight to the claims of other hypotheses, he and his co-workers [Klisiecki, Pickford, Rothschild and Verney, 1933] have thought that our knowledge of water diuresis is best interpreted as follows. The administration of water causes (probably through the mediation of the central nervous system) an inhibition of the secretory activity of the pituitary gland. The preformed pituitary hormone gradually disappears from the circulating blood, but for a time (about 30 min.) is present in sufficient concentration to restrain the activity of the kidney. As the concentration of pituitary hormone in the circulating blood diminishes, the rate of urine formation increases to a maximum. When as a result of diuresis much of the water excess is removed, the secretory activity of the pituitary gland is resumed, and the rate of urine formation diminishes.

Fee [1929] performed experiments on decerebrated hypophysectomized dogs, removing from his preparations all tissue known to secrete pituitary hormone. He found that these dogs did not develop spontaneous polyuria, and that a dose of water caused, after an initial delay, a diuresis which ceased when the excess of water was excreted. From this experiment it seems at first sight that the renal excretion of water may be controlled normally in the absence of any tissue known to be capable of secreting the pituitary hormone.

Fee's work was unfortunately ended by his untimely death and there remained some ambiguity in the interpretation of his results. First, a parenteral delay in the onset of diuresis is an important distinguishing feature of normal water diuresis, and from Fee's experiments one cannot eliminate the possibility that this delay is entirely of enteral origin. Indeed in his experiments a delay in the enteral absorption of water is not only possible but likely, for the water was given directly into the intestines through an abdominal incision. Laparotomy has been shown to cause delay in the alimentary absorption of water [Heller and Smirk, 1933]. Again, it must be decided whether the delay in diuresis might be due to incomplete recovery from the anæsthetic used for the decerebration, for both enteral absorption and renal excretion of water are delayed by anæsthesia [Heller and Smirk, 1932].

According to the pituitary hypothesis, the fall in the rate of urine formation which takes place when an excess of administered water is excreted is due to the secretion of an antidiuretic hormone by the pituitary gland. In the absence of this hormone a state resembling diabetes insipidus should be produced. Yet despite the absence of the pituitary gland in Fee's preparations the rate of urine formation returned to normal after excretion of the excess of water. But before interpreting this observation one must know if diuresis has ceased physiologically or because the animal is incapable of a water diuresis.

The following experiments were designed to overcome these and other difficulties in interpretation.

Methods.

The experiments were made on cats, and involved decerebration, with removal of the pituitary body, the collection of urine, and the administration of water. Each of these procedures was subject to modification and improvement throughout the earlier part of the work, and it is convenient to describe them separately.

Decerebration and hypophysectomy. Preparations made by the Sherrington method [1929] did not last sufficiently long in our hands, and this was abandoned early. The final routine was as follows: Anæsthesia was induced with ether by the open method, both carotid arteries ligated, and a tracheal cannula inserted. A flap of skin was turned back from the skull, the temporal muscle cleaned from the bone, and a half-inch trephine hole made in the left parietal region. The vault of the skull was then rapidly removed with bone forceps, and the meninges and brain stem severed with scissors flush with the bony tentorium. Simultaneously, the vertebral arteries were occluded by an assistant gripping behind the atlas vertebræ, so that no loss of blood occurred while the brain was scooped out with a teaspoon, and a clear view was obtained of the situation of the pituitary body. This was quickly and cleanly removed with a small gall-stone scoop, the sella inspected, swabbed, and tightly packed with cotton wool. The cranial cavity was packed with a piece of the detached temporal muscle over the cut surface of the brain and wads of cotton wool. The vertebral arteries were then released, and the flap of skin replaced. The anæsthetic was discontinued immediately the brain stem was severed, the total duration of administration having been between 10 and 15 min. from the time of beginning induction.

Collection of urine. A urethral cannula was introduced, after decerebration, exactly as described by Sherrington [1929]. This, however, involved cutting the symphysis pubis in two places, and by the time the operation was complete the preparation was no longer under the influence of ether: adequate local anæsthetization was tedious. It was found that sufficient of the urethra could be hooked up on curved forceps through a simple extraperitoneal supra-pubic stab in the mid-line, and the cannula inserted quickly and without hæmorrhage: the glass cannula (which just penetrated the internal sphincter) was later replaced by a rubber catheter, which reached into the bladder and did not damage the mucosa in the course of a long experiment. The wound was flooded with novocaine or percain during the operation.

Administration of water. This was at first given by one of two methods: into the stomach *via* a cannula tied into the œsophagus, or into the large intestine by a cannula tied into the slightly everted rectum. The cannulæ were closed off after the water had been given to prevent regurgitation. Both of these procedures involved considerable disturbance to the decerebrate animal, and it was felt that, although absorption of the water occurred in most cases, the life of the preparation had probably been shortened, and a variable amount of sensory stimulation produced which would militate against diuresis. It was found that by running the water from a pipette on to the back of the tongue, with the animal's head slightly raised, apparently unlimited quantities were swallowed quite normally. In this way up to 2 p.c. of body weight of water has consistently been given with no apparent ill effects, and the procedure repeated as many times as necessary.

During the experiment the preparation was kept on a warm stage, so that its temperature was usually 36–38° C. All wounds were swabbed with novocaine or percain. Blood-pressure records were not taken except on one or two occasions, but the condition of the animals as judged by reflexes (pinna, conjunctival, etc.) was good for 12–16 hours. As regards the pituitary gland, there is no doubt that this and all other hypophyseal tissue was removed completely. The brain-stem section passed through the mid-brain, and there was a clear space on the base of the skull between the plane of its cut surface and the sella turcica. The latter was thoroughly cleared, and the only possible situation left for pituitary tissue was in the vestigial connection of the anterior lobe with the roof of the mouth. Serial sections of the brain stump left after operation were made in one instance and histological examination revealed no pituitary gland tissue in it.

The method for estimating the rate of water absorption, where this was determined, is described by Smirk [1934].

Results.

The results of our early experiments were mostly negative for the animals usually had neither spontaneous polyuria nor diuresis after giving water, but as our technique developed (see "Methods") we had a greater measure of success. Spontaneous polyuria occurred in about half of our later experiments at times which varied from a half-hour or less to 5 hours after hypophysectomy. This increase in urine flow usually ceased spontaneously, within 1–3 hours, without serious depletion of the animals' water reserves (1·5 p.c. body weight or less being lost). When spontaneous polyuria did not occur a dose of water was given, and in most cases a diuresis resulted indicating that the kidneys themselves were still capable of a water diuresis. While trying to improve our methods it was found that when diuresis was absent after giving water this was often due to failure of alimentary absorption, but also that animals with satisfactory blood-pressure (over 120 mm. of mercury) and apparently in good condition might absorb water and yet have no subsequent increase in the rate of urine formation. In most animals, however, water absorption was followed, after a delay, by the usual rapid increase in urine flow, succeeded

by a decrease when the greater part of the excess of water was excreted. It is evident, therefore, that in these preparations there is nothing inherently adverse to water diuresis, but that sometimes unknown adverse factors are added which prevent the normal response to water administration.

In fifteen instances the administration of water has been followed after a delay by what appeared to be a typical water diuresis. In no case was the main increase in urine formation immediate, the average delay before the onset being 40 min., the time taken to reach the peak of diuresis averaging 126 min. The volume of urine excreted was less than the amount of water given and averaged 70 p.c.

The question as to whether this control of water elimination is truly physiological is in part answered by five experiments, in which absorption as well as excretion was recorded. In all of these experiments we employed the improved technique described under "Methods." The animals were deprived of food but were allowed a half-pint of milk on the evening preceding the experiment. All animals consumed their milk.

In the course of these five experiments we gave twelve doses of water and obtained twelve diureses. On ten occasions attempts were made to measure the water absorption times, and the measurements appeared to be satisfactory in eight of these attempts. In all of these eight instances the rate of urine formation was only just beginning to increase at a time when water absorption was thought to be complete (Fig. 1). In all eight instances the changes in the rate of urine formation lag behind the changes in the water load (*i.e.* the load of absorbed but as yet unexcreted water) often by as much as 20 min. (Fig. 2). In all of fifteen instances the increase in the rate of urine formation from the commencement of diuresis to the maximum urine flow was gradual, not immediate, and lasted on an average for 85 min. (Figs. 1, 2).

In all of fifteen instances where a sufficient time was allowed the rate of urine formation was observed to return towards normal on excretion of the greater part of the water given (Figs. 1, 2). In eight of these instances another diuresis was obtained after the first diuresis subsided, showing that the cessation of diuresis after the first dose of water was physiological in that the kidneys were still capable of a diuresis (Fig. 1). In all of six instances where the previous exhibition of a diuresis has demonstrated the capacity of the kidneys to have a water diuresis there is still a delay between the absorption of a second dose of water and the resulting diuresis; also the increase in the rate of urine formation to its maximum is gradual, not immediate. This indicates that the delay in the onset of diuresis and

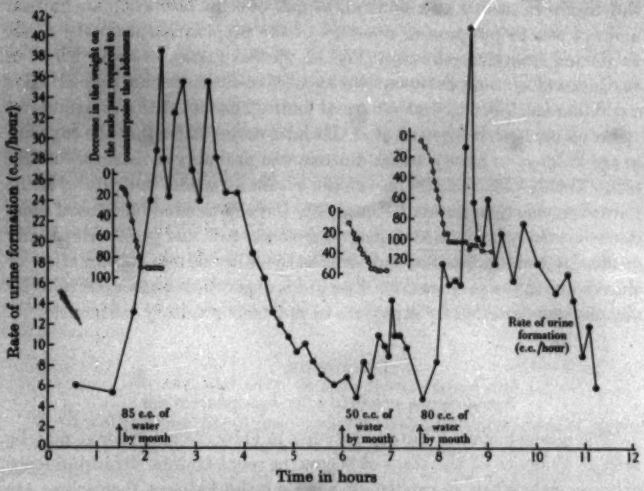
Rate of urine formation (c.c./hour)
Decrease in the weight on the scale pan required to counterpoise the abdomen
Rate of urine formation (c.c./hour)
85 c.c. of water by mouth
50 c.c. of water by mouth
80 c.c. of water by mouth
Time in hours

Fig. 1. The relationship between water absorption and excretion in decerebrated hypophysectomized cats.

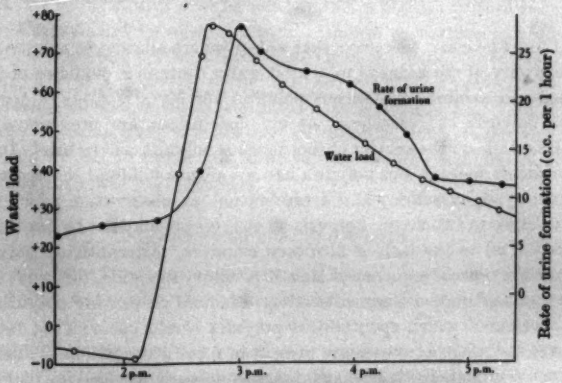

Fig. 2. The relationship between water load and diuresis.

the slow increase to a maximum in the rate of urine formation are natural and not due to progressing recovery of the preparation from the anæsthetic and from decerebration (Figs. 1, 2). Six instances occur where in the interval of time between two successive diureses the rate of urine formation has fallen to within normal limits. The fact that a diuresis after water occurs both before and after this interval indicates that the capacity of the kidneys to have a water diuresis was probably satisfactory at the time. Yet despite the absence of the pituitary gland the rate of urine formation was then normal (Figs. 1, 2). It was observed on several occasions that the urine formed during diuresis was pale and poor percentually in urea and chlorides. The flow was reduced by administering ether or chloroform to the preparation. Fee in his experiments observed that the diuresis was curtailed by injections of posterior pituitary hormone.

Discussion.

Spontaneous polyuria after hypophysectomy.

The absence of spontaneous polyuria in an acute experiment may be used as evidence of the state of the extra renal factors which influence polyuria only when it can be shown that the kidneys themselves are at that time capable of allowing such a polyuria to develop. But we have no means of determining whether the kidneys are capable of allowing spontaneous polyuria to develop, and in our experiments we have determined instead whether the kidneys are capable of a water diuresis.

It must be clear, therefore, that unless we are allowed to assume that the capacity of the kidneys to exhibit water diuresis is evidence of their capacity to exhibit spontaneous polyuria our investigations of spontaneous polyuria are valueless. As our conclusions are provisional the remarks made on the subject of spontaneous polyuria will be brief. In our experiments spontaneous polyuria has not always followed the operation of total hypophysectomy and decerebration, an observation which may be explained in two ways. Polyuria when it occurs may not be due (or not entirely due) to the lack of pituitary secretion. Alternatively polyuria may be the normal sequence of lack of pituitary secretion, but there may be present in some experiments adverse factors preventing polyuria, in the absence of which spontaneous polyuria would occur. That factors adverse to water diuresis are present in some preparations is evident, for many animals having no spontaneous polyuria are also incapable of responding by diuresis when a dose of water is administered to them. But

in other preparations where no polyuria occurred during the first 2–5 hours a subsequent administration of water indicated that the kidneys were capable of water diuresis. If the capacity for water diuresis is accepted as evidence of capacity to develop spontaneous polyuria, then it follows that the development of a spontaneous polyuria, of the diabetes insipidus type, does not depend solely upon a deficiency of pituitary hormone.

Again spontaneous polyuria if it does develop usually ceases spontaneously before there is serious depletion of the animals' water reserves. In five such instances the animals were then shown to be capable of water diuresis. Subject to the assumption made previously this suggests that some physiological mechanism not involving the pituitary hormone can restore the rate of urine formation to normal after spontaneous polyuria has developed.

Diuresis after water in hypophysectomized cats.

In all instances in this preparation where the administration of water is followed by diuresis, it resembles a water diuresis because there is a parenteral delay before its onset; the increase to the maximum rate of urine flow is ingravescent, not immediate; and the decline in urine flow to its basal rate is natural and not prolonged; also the volumes of urine secreted are not greater than the volumes of water given; the urine formed during diuresis is dilute and diuresis is curtailed by ether anæsthesia. As water administration elicits either a natural type of response or no diuresis (and because a natural response occurs much more frequently in the more perfect preparations) it seems justifiable to attribute the absence of a diuresis, where this occurs, to the presence in the preparation of factors which are adverse to water diuresis. Usually in imperfect preparations the absence of diuresis after giving water is explained by failure of the gut to absorb the water given, but in other cases the cause was undetermined.

Delay in the onset of diuresis.

In all of the fifteen instances where diuresis followed water administration there was a delay after giving water before the urine flow began to increase. In eight of these the water absorption times were measured, and it was found that absorption was always well in advance of diuresis; for as absorption ended the rate of urine formation was just starting to increase, the maximum urine flow being obtained about 80 min. later.

Now the usual interpretation of the pituitary hypothesis demands that the preformed pituitary hormone in the blood (or blood and tissues) should diminish and reach its minimum effective limit within 1 hour after the pituitary gland ceases to secrete. Therefore in animals deprived of their natural source of pituitary hormone for a period of 3–5 hours it would seem, according to this hypothesis, that the concentration of pituitary hormone should have already fallen below the concentration at which it is able to prevent water diuresis. In the absence of pituitary hormone the rate of urine formation, if not already high, would be expected to increase almost immediately, upon the absorption of water, to the maximal rate. But in these experiments it does not do so. The typical delay in onset and the further delay in attaining the maximum urine flow are still evident (Figs. 1, 2).

A potential flaw in the above argument is revealed by developing a slight modification of the usual pituitary hypothesis. Let it be supposed that the preformed pituitary hormone does not disappear spontaneously but that after the administration of water it is rendered inert by some process which is time consuming and might therefore account for the delay in diuresis. The delay in the onset of the first diuresis following the operation of hypophysectomy might be explained in this way. Delay in the onset of a second or a third diuresis in the same animal could not be so explained, for the preformed pituitary hormone once rendered inert can no longer be replaced by the secretory activity of the pituitary gland. Now in the five experiments where more than one diuresis was obtained in the same animal after hypophysectomy, the second or third diuresis showed no decrease either in the delay, in the onset of diuresis, or in the delay in attaining the maximum urine flow. It seems, therefore, that the delay in the onset of diuresis after giving water is, in this preparation, independent of the secretory activity of the pituitary gland and does not depend upon the presence of preformed pituitary hormone in the blood or tissues.

Furthermore, Verney and co-workers [1933] have shown that the onset of diuresis from the two kidneys does not always synchronize, and therefore a part of the delay must be located in the kidney. They discuss this as a serious but not insuperable objection to the pituitary hypothesis.

Ability to diminish the rate of urine flow.

The diminution in the rate of urine flow as the excess of water is excreted is to be taken as evidence that a natural control of diuresis is present which responds to the needs of the organism. One must suppose

that this ability to diminish the rate of urine formation is unconnected with the secretory activity of the pituitary gland, since this has been entirely removed. In experiments where only one dose of water is given it is fair to object that the diminished urine flow might be due to an inability of the kidneys to maintain diuresis. But in six diureses (in five experiments) this objection was eliminated by giving a further dose of water, obtaining a renewal of diuresis and in this way demonstrating the ability of the renal mechanism to respond to its proper stimulus.

In a few experiments a delay in the onset of diuresis and in attaining the maximum rate of urine flow was also obtained after the slow intravenous administration of 0·3 p.c. NaCl (Fig. 3). In all experiments where it was tried, the intravenous administration of urea or of hypertonic saline produced within a few seconds an increase in the rate of urine formation, the maximum rate being attained in the first urine sample which was collected after making the injection (see Fig. 3). Graphs showing the changes in the rate of urine formation are all peaked and the curves show no prolongation in their descents. Three or four successive peaks may be obtained in response to successive injections of urea.

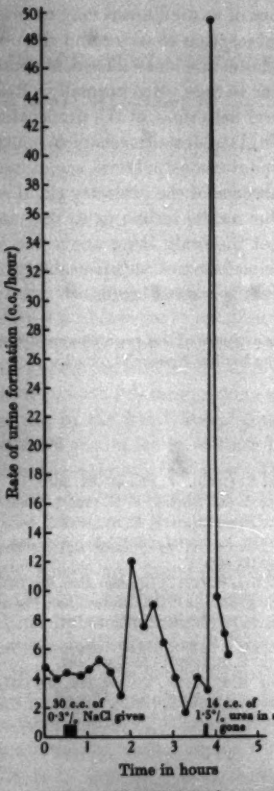

Fig. 3. The effect of giving intravenously 0·3 p.c. NaCl and 1·5 p.c. urea to decerebrated hypophysectomized cats.

We have as yet found no way of reconciling the results we have obtained with the pituitary hypothesis and our experimental observations on cats agree with and extend the results on dogs already published by Fee.

Summary.

In decerebrate cats with total hypophysectomy a natural control of the process of water diuresis may be present; there is the usual delay between the absorption of water and the onset of diuresis, and the increase to a maximum rate and subsequent decrease as the excess of water is excreted appear to take place normally. Reasons are given for believing that the natural behaviour of the preparation is due to the presence in it of the essential normal mechanism controlling water diuresis. The relation of the spontaneous polyuria sometimes encountered in this preparation to the absence of the pituitary gland and its hormone is discussed.

Our results incline us to the view that the pituitary gland and that part of the brain lying above the tentorium, which includes the hypothalamus, are not indispensable parts of the mechanism responsible for controlling water diuresis.

The expenses of this research were defrayed in part by a grant to one of us (W.H.N.) from the Medical Research Council.

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618.36:612.352.14

THE AUTOLYSIS OF THE PLACENTAL GLYCOGEN.

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(*Received February* 2, 1934.)

Claude Bernard [1859] showed by the iodine test that the rabbit's placenta contains glycogen. He assumed that it performs a glycogenic function for the fœtus until the fœtal liver is able to carry out that function for itself. Lochhead and Cramer [1908] supported this theory, showing that the glycogen is maximal in the placenta at the 21st–22nd day of pregnancy and diminishes from then until full term, during which period the glycogen is rapidly increasing in the fœtal liver. Harada [1916] stated that the placenta produces 40 mg. of lactic acid per 100 g. of tissue per day after removal from the uterus. Ishikawa [1928] determined the changes in glucose during autolysis of minced placentæ from full-term rabbits. He reported an increase in the glucose but no change in the lactic acid. No glycogen estimations were made. d'Piane [1926] perfused human placentæ with glucose and found glycogen was formed. Huggett [1929] found that the glycogen in the placenta of the rabbit was very stable despite metabolic and pharmacological experiments which in the maternal liver altered the percentage present and despite the fact that the glycogen was mainly present in the decidual (maternal) tissues of the placenta. The placental glycogen therefore appears to resemble muscle glycogen in its metabolism, being much less easily affected than the liver glycogen by starvation, carbohydrate feeding, adrenaline and insulin injections. It is in fact only altered to any appreciable degree by an excessive increase in endogenous katabolism.

In the liver glycogen on autolysis yields glucose; in muscle on hydrolysis it yields lactic acid [Parnas and Wagner, 1914]. It became therefore of interest to determine quantitatively the products of autolysis of the placental glycogen. The results obtained indicate a very small formation of lactic acid confirming Harada [1916], and that the glycogen present

yields nothing but carbohydrate, of which approximately two-thirds is glucose and one-third polysaccharide. In other words, the autolytic changes in placental glycogen resemble those in the liver rather than those in muscle. The placenta therefore probably has a similar glycogenic function as Bernard suggested, and the glycogen in it does not serve as a store of energy for the placenta itself as the glycogen in muscle does for the muscle.

Methods.

The placental glycogen in the rabbit is maximal at the 21st–22nd day of pregnancy and is present almost entirely in the maternal (decidual) portion of the placenta. Consequently, the rabbits were killed at the 21st day from being served with the buck and the maternal part of the placenta separated from the fœtal. The determinations were carried out on the former, though in certain experiments the effects of autolysis were determined on the whole placenta, that is, on the maternal in the presence of the fœtal portion.

The placenta was obtained from the rabbit by the technique described by Huggett [1929], and after removing blood with filter paper, weighed and ground up with sand as quickly as possible. The pulp so formed was divided into two parts, A and B. Portion A was mixed with three times its volume of absolute alcohol for estimation of glycogen, reducing sugar and lactic acid content. Portion B was mixed with an equal volume of 0·9 p.c. sodium chloride, a little toluene added and the whole incubated at 37° C. for 24 hours. After incubation it was treated with ice-cold absolute alcohol and the same estimations were made as on portion A.

Determination of glucose and lactic acid. In order to extract these, following the method of Anderson and Macleod [1930], three volumes of absolute alcohol were used for the first extract, 75 p.c. alcohol for the second and third. The mixed extracts were evaporated, the glucose precipitated with copper sulphate and lime and then released with sulphuretted hydrogen. The glucose was determined by the method of Hanes [1929, modification of Hagedorn and Jensen's method]. The lactic acid was determined by the method of Friedemann, Cotonio and Shaffer [1927].

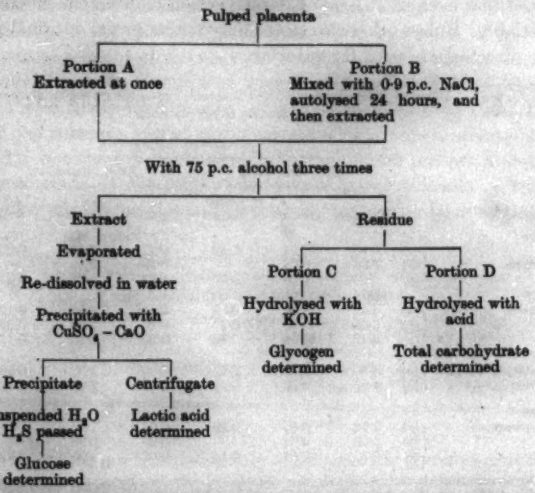
Determination of glycogen. Pflüger's method was used but was modified by adopting the neutralization of the potash hydrolysate with glacial acetic acid as advocated by Evans, Tsai and Young [1931]. Further, 75 p.c. alcohol was used instead of 66 p.c. to precipitate the glycogen. Apart from these modifications the estimation was performed

throughout in centrifuge tubes as in the previous determinations of placental glycogen [Huggett, 1929].

Determination of total carbohydrate. This was done when necessary on the ground-up placenta before and after autolysis and also on the residues after extraction with 75 p.c. alcohol and removal therefore of sugar. The tissue or residue was hydrolysed for 5 hours with hydrochloric acid under a reflux condenser. The solution was neutralized with sodium carbonate, filtered and the proteins precipitated with zinc hydroxide. The glucose formed was estimated by the same method as in the alcoholic extracts.

These procedures are summarized in Table I.

TABLE I.



The difference between portions C and D is a measure of the carbohydrates of size intermediate between glycogen and sugars.

Experimental.

In the previous determinations the total carbohydrate content of the residue after triple extraction with alcohol (portion D of Table I) was not estimated. The results expressed in Tables II and IV–VI therefore show the glucose and glycogen quantities as percentages of the original placental weight and the quantity of carbohydrate recovered as a percentage of the initial glycogen. Table III shows that the total carbohydrate content of

the placenta is unchanged on autolysis, for the estimations on portion D show that there is an appreciable quantity of carbohydrate of molecular size intermediate between glycogen and sugars and that this is equivalent to the unrecovered portion of Tables II and IV–VI.

Experiments (*a*), (*b*), (*c*), etc., indicate separate experiments on different placentæ from different animals. Experiments (i), (ii), (iii), etc., indicate separate experiments on the same animal. All the analyses have been carried out in duplicate and the figures given represent the average of the duplicate results; they agreed to within 2 p.c. in the glucose and glycogen estimations and to within 6 p.c. in those of lactic acid. Toluene was added to the autolysate in all experiments, since a preliminary test revealed that even in 24 hours bacterial action reduced the glucose yield appreciably. Unless otherwise stated only the maternal decidual portion of the placenta was used for autolysis.

TABLE II. Effect of autolysis (aerobic and anaerobic) upon the glycogen, glucose and lactic acid content of the placenta.

		Percentages of the placenta						
		Before incubation			After incubation			Percentage of total initial carbohydrate recovered
Remarks		Glycogen	Glucose	Lactic acid	Glycogen	Glucose	Lactic acid	
Decidual portion:								
Aerobic autolysis	(*a*)	6·40	0·04	0·12	0·47	2·62	0·20	44·9
	(*b*)	3·96	0·04	—	0·19	2·19	—	55·3
	(*c*)	4·40	0·05	0·23	0·22	1·98	0·21	45·7
Decidual portion:								
$^{*}O_2$ gas bubbled through autolysate	(*a*)	4·48	0·04	0·12	0·43	2·36	0·12	58·7
	(*b*)	6·65	0·05	0·17	0·23	2·60	0·38	39·1
	(*c*)	4·12	0·03	0·16	0·25	1·73	0·12	44·5
Decidual portion:								
Anaerobic autolysis N_2 gas bubbled during autolysis	(*a*)	4·50	0·00	0·26	0·45	1·97	0·33	62·5
	(*b*)	4·67	0·03	0·11	0·03	2·24	0·16	44·8
Whole placenta:								
Aerobic autolysis with fluoride	(*a*)	1·74	0·05	0·16	0·06	1·10	0·18	59·4
	(*b*)	1·76	0·04	—	0·05	0·99	—	52·8
	(*c*)	2·87	0·04	0·12	0·09	1·98	0·12	66·0

* In these three experiments carbon dioxide was collected in soda-lime tubes, none was detected throughout autolysis, despite acidification afterwards with tartaric acid in the third experiment.

A. *Autolysis, aerobic and anaerobic.* Portion B (Table I) suspended in saline was incubated for 24 hours at 37° C. In the first set of experiments autolysis proceeded alone; in the second set oxygen was bubbled through the whole time and the carbon dioxide formed was trapped in weighed soda-lime tubes; while in the third set nitrogen was bubbled through instead of oxygen to give anaerobic autolysis.

In all these the results obtained were essentially the same; lactic acid was practically unchanged, glycogen disappeared almost entirely and

glucose was formed to the extent of approximately 50–60 p.c. of the original glycogen. No carbon dioxide was formed (except in one experiment, which was not confirmed on repetition); this was despite acidification with tartaric acid. The result is to be expected, since autolytic changes in general are accomplished by hydrolysis and not by oxidation.

The results on whole placentæ show a slightly higher percentage of recovered carbohydrate and a lower initial percentage of glycogen. The latter is due to the fact that the maternal portion of the placenta alone contains the glycogen, and this is mixed with the fœtal portion in the autolysis. The higher recovery of carbohydrate is apparently due to the dilution of the autolysate. This is confirmed by the effects of autolysis seen when maternal placentæ are diluted (Table IV).

In all these experiments no determination was made of the total carbohydrate content of the placental residue after the alcoholic extraction of the glucose and lactic acid, that is, portion D in Table I. The residue was used solely for glycogen estimation (portion C in Table I.) In view of the constant loss of carbohydrate which formed approximately one-half to one-third of the total initial carbohydrate present, determinations were made on the total carbohydrate in the placenta before and after hydrolysis (Table III) and on the residue (portion D) after alcoholic extraction of the autolysate.

TABLE III. Percentage recovery of initial carbohydrate on autolysis and determination of total carbohydrate.

	Percentage of total carbohydrate present		Percentage of initial carbohydrate recovered
	Before autolysis	After autolysis	
(*a*)	2·58	2·39	93
(*b*)	2·84	3·10	109
(*c*)	2·98	2·92	98

The total carbohydrate content of the placenta is therefore practically unchanged by autolysis.

On determining the total carbohydrate present in the residue (portion D) it was found to exceed the glycogen very much and to form 35 p.c. of the glucose formed in the case of the whole placenta and 50 p.c. of the glucose formed where only the maternal (decidual) portions were hydrolysed. These figures mean that the carbohydrate lost and not detected as glycogen or glucose is present as an intermediate carbohydrate product, probably a polysaccharide. The question was further investigated by taking the residue containing glycogen, after extraction of the sugars with 75–80 p.c. alcohol, and further extracting it with 66 p.c. alcohol, this

yielded a milky solution which on acid hydrolysis gave a reducing sugar. The milky solution so obtained also formed a slight precipitate on saturation with ammonium sulphate but gave no significant colour with iodine.

It seems permissible on this evidence to conclude that the residue contains glycogen together with intermediate products of its hydrolysis resembling dextrin. To determine exactly the kind of dextrin would require work with larger amounts of placenta than are at our disposal at the moment. In all probability it is a mixture of dextrins that is formed and not a single polysaccharide.

B. *The effect of reaction upon placental autolysis* was investigated under the following headings:

(*a*) Change of reaction in the unbuffered solution. This was very slight, only altering from about *p*H 7·15 to 7·4 (determined colorimetrically).

(*b*) Autolysis when buffered at *p*H 5·0, 7·0 and 9·0. Buffering was maintained with the universal buffer of Prideaux and Ward [1924] except in one experiment where the buffer used was ammonium acetate.

(*c*) Dilution of the autolysate. This was accomplished by using saline and no buffer solutions.

TABLE IV. Effect of buffering and of dilution of autolysate on carbohydrate autolysis in the decidual portion of the placenta.

Remarks		Percentages of the placenta (decidual)				Percentage of initial carbohydrate recovered
		Before incubation		After incubation		
		Glycogen	Glucose	Glycogen	Glucose	
Autolysis at *p*H 7·0:						
Ammonium acetate buffer	(*a*)	3·30	0·03	0·26	1·96	62·2
Universal phosphate buffer	(*b*)	4·01	0·05	0·43	3·20	83·8
	(*c*)	3·44	0·03	0·18	2·40	68·7
	(*d*)	5·90	0·06	0·40	3·39	59·3
8 hours' autolysis at *p*H 5·0, 7·0 and 9·0:						
(*e*) (i)		4·55	0·06	1·23	1·06	47·5
(ii)		4·55	0·06	1·29	1·68	61·5
(iii)		4·55	0·06	1·32	1·50	58·3
Effect of dilution of autolysate:						
(*f*) (i) With 2 vol. of saline		*	0·04	0·02	2·54	—
(ii) With 4 vol. of saline		*	0·04	0·01	2·76	—
(*g*) (i) With 1 vol. of saline		3·21	0·06	0·12	1·84	55·5
(ii) With 4 vol. of saline		3·21	0·06	0·10	2·25	68·6

* The glycogen estimations here were accidentally lost.

Solutions buffered at *p*H 7·0 yield about 10–15 p.c. more glucose than in the unbuffered state. This is probably not due to the phosphate (universal) buffer used reacting with the carbohydrate, as occurs in

muscle metabolism, since it was obtained with the ammonium acetate buffer. It may possibly be due to the maintenance of constancy of reaction; but even in unbuffered autolysis the reaction does not materially alter. Where the medium was buffered to an acid, reaction glucose formation is markedly inhibited, and yet glycogen is broken down if anything more rapidly. In the alkaline buffer the glucose formation is only a little less pronounced than at *p*H 7·0.

Dilution of the autolysate without buffering yields rather more glucose. This result is in line with the results of autolysis of whole placentæ where more glucose was obtained than when maternal placentæ alone were autolysed.

C. *Effect of time on the products of autolysis.* Autolysis was allowed to proceed for periods varying in different experiments between 4 hours and

TABLE V. Effect of time of autolysis upon the glycogenolysis of the decidual portion.

	Percentages in the placenta				Percentage of initial glycogen recovered as glucose	Percentage of initial carbohydrate recovered
	Before incubation		After incubation			
	Glycogen	Glucose	Glycogen	Glucose		
Autolysis in unbuffered solutions:						
(*a*) (i) 12 hours	—	0·04	0·14	1·33	—	—
(ii) 48 hours	—	0·04	0·00	2·63	—	—
(*b*) 70 hours	4·4	0·05	0·05	2·38	50·0	50·3
Autolysis when buffered at *p*H 7·0:						
(*a*) 4 hours	5·9	0·06	2·76	1·51	22·9	69·7
(*b*) 8 hours	5·9	0·06	1·83	2·29	35·0	66·2
(*c*) 12 hours	5·9	0·06	0·68	2·53	39·0	50·5
(*e*) 24 hours	5·9	0·06	0·40	3·39	56·4	59·3

70 hours both in buffered and unbuffered solutions. The results are exemplified by Fig. 1.

The percentage of glucose formed from glycogen increases as autolysis proceeds. The formation of glucose takes place more slowly than the breakdown of glycogen. When equilibrium was reached in each case not more than 50–60 p.c. of the glycogen originally present was recovered in the form of glucose.

These results emphasize the need for a rapid removal of the placenta after killing, since autolysis proceeds rapidly in the early stages.

D. *The autolytic enzyme.* The properties of the autolytic enzyme are important in comparing the functions of glycogen in the placenta and in muscle. Meyerhof [1921] showed that fluoride inhibited lactic acid formation in muscle, and Evans [1922] showed the same thing for the glycolysis in blood. Since lactic acid formation is a relatively unimportant

feature in the glycogenolysis of the placenta, we should not expect fluoride to affect materially the breakdown of glycogen. This is illustrated as the case by the result in Table II. There was no material alteration in the glycogen breakdown or lactic acid formation although rather more glucose was formed. This might be due to some inhibition of glycolysis.

The second point about the enzyme is the fact noted above, that when autolysis is allowed to proceed, equilibrium is reached in about 24 hours.

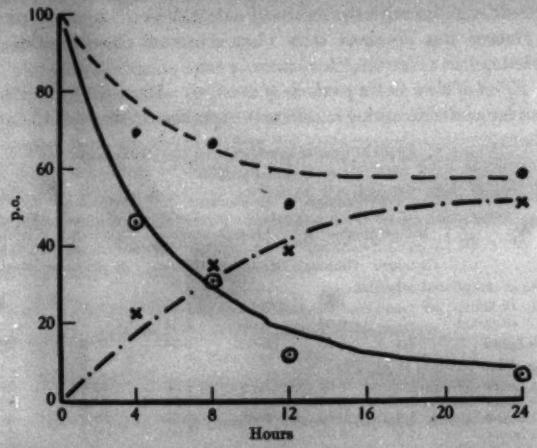


Fig. 1. Effect of time on the autolysis of placental tissue. ⊙—⊙ Percentage of initial glycogen recovered as glycogen. ×—·—× Percentage of initial glycogen recovered as glucose. ●—● Percentage of initial glycogen and glucose recovered as glycogen and glucose.

The third point of interest is the origin of the enzyme. Is it resident in the placental tissues or does it reach it *via* the maternal blood from some organ such as the pancreas?

In an attempt to solve this point the placenta was rendered blood-free by the vivi-perfusion method of Bogniard and Whipple [1932]. The rabbits were then killed. The placentæ thus obtained contained no maternal blood, and, as the maternal portion only was used, contained no fœtal blood. The essence of the method is to allow Ringer-Locke solution to run in at the jugular vein and simultaneously blood to run out at the carotid artery. Adrenaline was injected towards the end of the experiment

to increase the blood-pressure and so to promote blood flow. This is allowable, since it has been shown [Huggett, 1929] that adrenaline has no effect on the placental glycogen. In about 30 min. the fluid leaving the carotid was colourless.

Table VI shows that the quantity of carbohydrate recovered is the same whether whole placentæ or only maternal placentæ are used, and is

TABLE VI. Effect of removal of maternal blood before killing on the autolysis of the maternal portion and the whole placenta.

	Before incubation		After incubation		Percentage of initial carbohydrate recovered
	Glycogen	Glucose	Glycogen	Glucose	
Maternal (decidual) portion of the placentæ:					
(*a*) Almost blood-free	4·04	0·01	0·06	2·16	51·0
(*b*) Blood-free	3·92	0·08	0·16	2·20	54·3
Whole placentæ:					
Blood-free	2·48	0·07	0·05	1·34	49·6

the same when the blood is washed out as when it is not. In other words, the amylase is seated within the decidual cells of the placenta, and is not merely in the maternal blood.

DISCUSSION.

The products of autolysis of the placental glycogen are entirely carbohydrates, the only lactic acid produced in 24 hours being traces of the order described by Harada [1916]. The carbohydrates formed fall into two classes, soluble and insoluble in 66 p.c. alcohol. The former includes the reducing sugars of which the chief appears to be glucose. The only osazone obtained was glucosazone. There was no sign of maltosazone or of the osazone of Barbour's trisaccharide [1929]. There was, however, a small quantity of something else present, since estimations on two samples of the reducing power after hydrolysis of the alcohol-soluble fraction with acid gave higher results than when no such hydrolysis was carried out. This small quantity would, however, only account for 4 p.c. of the original glycogen.

The alcohol-insoluble portion contained only a trace of glycogen but sufficient extra carbohydrate to account for the remaining glycogen. This appears to be a polysaccharide of molecular size less than that of glycogen but yielding no significant colour with iodine.

Aerobic and anaerobic autolysis yield the same results and no carbon dioxide formation in either case. Increased acidity up to *p*H 5·0 does not inhibit glycogen breakdown, but it does decrease the formation of glucose.

Increased alkalinity up to *p*H 9·0 has little or no effect on either process. Phosphate and fluoride ions have no gross effect on placental autolysis. This compares with muscle autolysis [Meyerhof, 1921].

Sahyun and Alsberg [1931] have observed that in hydrolysis of pure liver glycogen with takadiastase equilibrium is reached when only 52 p.c. of the glycogen broken down is present as reducing sugars. They state that in addition to the monosaccharide formed a polysaccharide is present. In this respect therefore there appears to be some parallel between the autolytic hydrolysis of glycogen in the placenta and the hydrolysis by takadiastase of glycogen in the liver. Lochhead and Cramer [1908] extracted with glycerol from the placenta an enzyme which converted pure glycogen into glucose. The glycogen used had not, however, been prepared from the placenta.

These experiments therefore confirm Claude Bernard's original view that the placenta serves as a store of carbohydrate for the fœtus, and they offer no evidence of parallelism of function between muscle and placental glycogen. Taken in conjunction with previous experiments [Huggett, 1929] the decidual glycogen appears to be nearly independent of the maternal metabolism, to be remarkably stable and to be controlled mainly by the fœtal demands although present in the maternal (decidual) tissues of the placenta.

Summary.

1. Autolysis of minced rabbit's placenta results in disappearance of the glycogen and its replacement by approximately two-thirds of glucose and one-third of a dextrin-like polysaccharide.

2. No carbon dioxide and only traces of lactic acid were found.

3. The same result was obtained with whole placentæ and decidual portions only, and was unaltered by the absence of oxygen, by fluoride or phosphate, or by previous removal of maternal blood.

4. Alteration in reaction did not alter the disappearance of glycogen, but more glucose was formed at *p*H 5·0 than at *p*H 7·0.

Our thanks are due to Dr Arthur Wormall of this department for many helpful suggestions.

We are indebted to the Government Grant Committee for a grant to one of us (A. St G. H.) which has partly covered the expenses incurred.

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REFLEXES IN THE TRICEPS EXTENSOR PREPARATION OF THE FORELIMB.

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(*Received February* 5, 1934.)

In a recent study of forelimb reflexes [Miller, 1931] it was shown that coordination is usually reciprocal in those evoked by ipsilateral nerve stimulation: thus, associated with the contraction of biceps brachii in the flexor reflex there is generally inhibition of the lateral head of triceps. Sometimes, however, in the course of the flexor reflex a considerable amount of contraction occurs in triceps, being interspersed with phases of inhibition. It was, therefore, decided to investigate further the conditions favouring respectively reflex excitation and inhibition in triceps and, if possible, to determine the factors leading to the conversion of one process into the other; this led eventually to the study of a number of other triceps reflexes.

The vasto-crureus muscle of the hindlimb had been observed by Sherrington and Sowton [1911] to respond with excitation or inhibition according to the kind of afferent nerve stimulation used: favourable to excitation were brief galvanic currents, particularly those furnished by a rotating rheonome; acting in the same direction was weak faradization, which, on intensification, evoked powerful inhibition.

In the present investigation the results on the triceps extensor of varying the intensity of faradic stimulation were particularly studied. In general it was found, in the case of the median and ulnar nerves, that faradic stimulation of low intensity induced excitation, whilst that of greater intensity yielded responses showing considerable inhibition. A somewhat similar observation had been made by Socin and Storm van Leeuwen [1914]; these workers, however, used the complete triceps brachii, which is in reality of mixed extensor-flexor function, since its

caput longum is in part a potential shoulder flexor; it is thus not clear from their observations whether they had dealt with reflex reversal in the true sense. In order to obviate any such uncertainty in my own experiments the influence of the caput longum was eliminated from the triceps by section of its motor nerve or, occasionally, by tenotomy; the preparation resulting constituted the "triceps extensor," which, for brevity, will often be referred to in this article as simply the "triceps."

Methods.

The cat was anæsthetized with chloroform and ether and tracheotomy was performed. The right brachial plexus was exposed by an axillary incision and all nerves were severed with the exception of the radial, median, ulnar and medial cutaneous; the median and ulnar nerves were later severed at the elbow joint; the dorsal interosseous nerve was cut at the elbow to paralyse the extensor muscles of the forearm. In the initial experiments the long head of triceps was rendered ineffective by detaching its tendon from the olecranon; in the majority of experiments recourse was had to severance of the radial nerve branches at their points of entry into the caput longum: to effect this the long head was dissected away from the lateral head until its entering nerves were visible at a level of 2·5–3 cm. below the metacromion. The remaining effective parts of triceps, namely the lateral and medial heads, exert on the olecranon an influence purely extensor or "anti-gravity" in nature. The spinal cord was exposed in the upper lumbar region preparatory to section, which was sometimes performed either prior to, or during the progress of, recording; in the latter case comparisons could be made of the responses of triceps before and after cord section.

Decerebration was performed by the trephine method, the plane of transection lying immediately in front of the superior colliculi or through the front parts of these structures. For recording the animal was placed on its left side, the right humerus being held upright by a clamp embracing its shaft, the triceps thus being pulled upon by the weight of the forearm. A wide, flat-jawed swivel clamp was designed to grip the spine of the scapula and another clamp was applied to the spines of two upper lumbar vertebræ. The various clamps were supported by uprights secured to the table top and effective immobilization was thus attained.

The transmission of triceps movements was by a thread from the olecranon, passing over a pulley to a crank lever pulling against a light, coiled spring. The magnification of the lever was 4·7 times; the tension

with the recording arm horizontal amounted to 8·5 g.; an excursion of 1 cm. upward or downward of the writing point corresponded to an addition or subtraction respectively of 100 mg. Recording, which was on the Brodie-Starling kymograph, was begun at an interval of about 4 hours after decerebration, at which time the triceps reflexes had become very brisk.

The stimulating currents for the nerves of the two sides were derived from two special inductoria of the kind previously employed [Miller, 1931]; both were made by Messrs C. F. Palmer (London), Ltd. Each was operated by an accumulator with a rheostat and ammeter in the primary circuit, the current flow thus being kept constant. The electrodes were of the Sherrington [1909*b*] side-tube pattern, the bipolar terminals being of silver wire; in a few cases simple hand electrodes were used. Faradization was begun and ended by the operation of a secondary short-circuit key, the second blade of which controlled a signal magnet.

Results.

(1) *Actions of the afferent nerves of the forelimb on the triceps extensor preparation.*

Medial cutaneous nerve. For the purpose of stimulation this nerve was isolated as one or more filaments on the medial side of the elbow on its course to the skin of the forearm; it is an example of a purely cutaneous nerve. Under the conditions of recording in these experiments, when the tonus of triceps is intense, the ipsilateral medial cutaneous nerve usually exerts a predominantly excitatory effect on the triceps. With a weak current the ascent of the myogram is moderately steep, the "ascent-plateau turn" is rounded and the "plateau" [Liddell and Sherrington, 1924*a*] declines slightly; sometimes both ascent and plateau show oscillations; the plateau is followed by a rounded "angle" [Fulton, 1926] with moderate after-discharge (Fig. 1 A). With stronger stimulation the ascent is prompter and higher, a "réaction d'emblée" [Liddell and Sherrington, 1924*b*]; the plateau is rising, horizontal or slightly declining; the angle is sharper with after-discharge (Fig. 1 B). The ipsilateral medial cutaneous reflex adds itself to the crossed extensor response (Fig. 2), the sum of crossed and uncrossed effects being much greater than that from the ipsilateral nerve alone; the superposed ipsilateral contraction is closely similar to that evoked by itself. Resembling these results are those of mechanical stimulation of the nerve, as by a pinch, which yields effects mainly excitatory in nature (Fig. 3).

After cutting the spinal cord in the lumbar region triceps tone is much augmented and positive reflex effects continue: here, besides oscillations

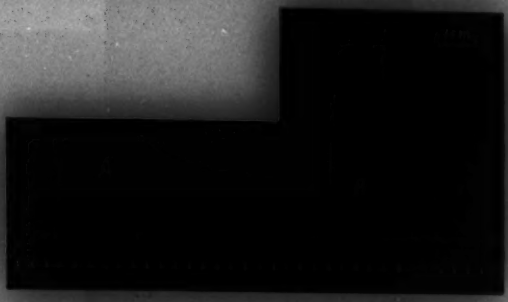

Fig. 1. In this and all figures, reflex myograms of triceps extensor preparation, upward movement of writing point signifies contraction, downward movement, relaxation. Signal in this figure, faradization of ipsilateral medial cutaneous nerve. A, sec. dist. 400 mm.; B, sec. dist. 200 mm. Time in all figures, 0·5 sec. Contraction and after-discharge.

Fig. 2. Upper signal, faradization of ipsilateral medial cutaneous nerve: sec. dist. 200 mm. Lower signal, faradisation of contralateral ulnar nerve: sec. dist. 400 mm. Superposed ipsilateral reflex.

of the plateau, there is sometimes positive rebound (Fig. 4). With strong stimulating currents there is no reversal to inhibition, as often occurs in

Fig. 3. Pinch with forceps of ipsilateral medial cutaneous nerve. Mainly contraction.

Fig. 4. Spinal cord cut in upper lumbar region. Faradization of ipsilateral medial cutaneous nerve. Sec. dist. 200 mm. Contraction and positive rebound.

Fig. 5. Spinal cord cut, same experiment as Fig. 4. Faradization of ipsilateral medial cutaneous nerve. A, sec. dist. 150 mm.; B, sec. dist. 100 mm. Declining plateaux with clonus.

the case of median and ulnar nerves (*vide infra*); the plateau is, however, often declining (Fig. 5 A) and may be complicated by clonus waves (Fig. 5 B).

Fig. 6. Upper signal, faradization of ipsilateral medial cutaneous nerve: sec. dist. 200 mm. Lower signal, faradization of contralateral ulnar nerve: sec. dist. 400 mm. Same currents in A and B. A, inhibition of crossed extensor reflex; B, contraction in after-discharge of crossed reflex.

Fig. 7. Same experiment as Fig. 6. Upper signal, faradization of ipsilateral ulnar nerve: sec. dist. 200 mm. Lower signal, faradization of contralateral ulnar nerve: sec. dist. 400 mm. Same currents in A and B. A, inhibition of crossed extensor reflex; B, inhibition of after-discharge of crossed reflex.

With a moderate amount of tone in triceps medial cutaneous may yield inhibition of the tone of a crossed extensor reflex (Fig. 6 A), though the same stimulus yields contraction during the after-discharge of a similar crossed reflex (Fig. 6 B). Sometimes, from the resting position

of the muscle, medial cutaneous yields inhibition only, followed by rebound. It is interesting to compare with these responses the action of ulnar nerve, which, to the same stimulus as in Fig. 6, yields relatively more powerful inhibition of the crossed extensor plateau and also clear inhibition of its after-discharge (Fig. 7 A and B).

To test further the action of the ipsilateral medial cutaneous nerve on the triceps it was examined without recording in the decerebrate preparation; the nerve to the caput longum was cut, also the musculocutaneous nerve, thus paralysing all the flexors of the limb. Under these conditions medial cutaneous nerve yields, so far as one can judge by inspection and palpation, only inhibition of triceps, which is followed by positive rebound; also, on direct inspection of the lateral head of the muscle, no contraction is visible.

Fig. 8. A, faradization of ipsilateral superficial radial nerve: sec. dist. 350 mm. Contraction and positive rebound. B, upper signal, faradization of ipsilateral superficial radial nerve: sec. dist. 350 mm.; lower signal, faradization of contralateral ulnar nerve: sec. dist. 350 mm. Contraction in descent of crossed extensor reflex.

Superficial radial nerve. This nerve was isolated for stimulation at the elbow just beyond its place of emergence between the lateral head of triceps and brachialis muscle; like the medial cutaneous it is a nerve of purely cutaneous distribution. The superficial radial nerve usually yields excitatory effects when the triceps is initially in the resting condition: there is a prompt contraction, then a brief relaxation and a plateau either ascending or descending; stimulation is followed by a slight positive rebound; this is then followed by what appears to be after-discharge (Fig. 8 A). Similar excitatory effects may be manifested during the descent from a crossed extensor reflex (Fig. 8 B). In other cases some degree of inhibition is yielded during subsidence of crossed extension (Fig. 9 A). Inhibition is yielded more clearly to the same stimulus during the stimulation period of the crossed extensor reflex

(Fig. 9 B); the identical stimulus may, however, have yielded excitation a few seconds earlier when the muscle was in a relaxed state. On testing the superficial radial nerve in the decerebrate preparation, without recording, the only effects detectable are inhibition of triceps during the stimulus, followed by positive rebound.

Fig. 9. Upper signal, faradization of ipsilateral superficial radial nerve: sec. dist. 200 mm. Lower signal, faradization of contralateral ulnar nerve: sec. dist. 400 mm. Same currents in A and B. A, inhibition of after-discharge of crossed extension; B, inhibition of plateau of crossed extension.

Fig. 10. Upper signal, faradization of ipsilateral dorsal interosseous nerve: sec. dist. 300 mm. Lower signal, faradization of contralateral ulnar nerve: sec. dist. 300 mm. Same currents in A and B. A, inhibition of plateau of crossed extension; B, inhibition of after-discharge of crossed extension.

Dorsal interosseous nerve. Stimulation was applied to this nerve just above its entrance into the muscles of the forearm; its afferent fibres are mainly proprioceptives. It revealed itself in a somewhat limited study solely as inhibitory of triceps: this is shown both during a crossed extensor reflex (Fig. 10 A), as well as in the declining phase of the same

reflex (Fig. 10 B); there is in this latter reaction an absence of rebound. A similar inhibitory response was obtained when direct observations were made without recording. The dorsal interosseous does not, in the limits of my observations, yield a positive response from the resting position of the muscle, as happens in the case of superficial radial.

Median and ulnar nerves. These nerves are mixtures of cutaneous afferent and proprioceptive fibres; being very similar as to mode of distribution, the results of their stimulation may logically be considered together. Since stimulation may be more conveniently applied to the ulnar most of the results were obtained from this nerve; they do not differ materially from those from the median nerve. Stimulation was applied to each nerve in the neighbourhood of the elbow.

Fig. 11. Upper signal, faradization of ipsilateral ulnar nerve: sec. dist. 250 mm. Lower signal, faradization of contralateral ulnar nerve: sec. dist. 250 mm. Same currents in A and B. A, inhibition of plateau of crossed extension; B, inhibition of after-discharge of crossed extension.

The most usual result of faradization of the ipsilateral ulnar nerve, the spinal cord being intact, is inhibition of the tone of triceps; the inhibition is best displayed against the background of tonus occurring either during the excitation period of a crossed extensor reflex or during its after-discharge: in the first case the inhibition is shown as a temporary subsidence of the tonus plateau (Fig. 11 A), in the second as a sharp fall in its after-discharge (Fig. 11 B). Inhibition is also demonstrable in the resting triceps, its amount varying with the state of the muscle: thus, after induction of the "shortening reaction" [Sherrington, 1909*a*] inhibition is extensive and is followed by rebound (Fig. 12 A): after the

"lengthening reaction" [Sherrington, 1909*a*] inhibition is but slight, rebound scarcely noticeable (Fig. 12 B). Sometimes a small contraction precedes the inhibition, which is followed by large rebound (Fig. 13).

After section of the spinal cord in the upper lumbar region there follows in triceps a great augmentation of tone, which often waxes and

Fig. 12. Faradization of ipsilateral ulnar nerve: sec. dist. 250 mm. A, greater inhibition after shortening reaction, *S.R.* B, smaller inhibition after lengthening reaction, *L.R.*

Fig. 13. Upper signal, faradization of ipsilateral ulnar nerve: sec. dist. 250 mm. Lower signal, faradization of contralateral ulnar nerve: sec. dist. 400 mm. Small contraction followed by inhibition and rebound.

wanes spontaneously. As compared with the effects before cord section there is now, to a given weak stimulus, reflex reversal: this is shown in a prompt contraction with rising plateau, succeeded by after-discharge; both phases show considerable clonus (Fig. 14 A). A weaker current yields a similar positive response of reduced amplitude, indicating that the stimulus is approaching threshold value (Fig. 14 B).

Fig. 14. Spinal cord cut. Faradization of ipsilateral median nerve. A, sec. dist. 400 mm.; B, sec. dist. 480 mm. Contraction, clonus, after-discharge.

Fig. 15. Continuation of Fig. 14. Faradization of ipsilateral median nerve. A, sec. dist. 350 mm.; B, sec. dist. 300 mm. Increasing inhibition and rebound.

Fig. 16. Continuation of Fig. 15. Faradization of ipsilateral median nerve. A, sec. dist. 250 mm.; B, sec. dist. 200 mm. Decreasing contraction; increasing inhibition and rebound.

On increasing the current intensity a small amount of inhibition succeeds the initial contraction: there follows slight rebound; then there is after-discharge, apparently the exact equivalent of that with the weaker stimulus and probably to be regarded as the prolongation of the phase of contraction during the stimulus (Fig. 15 A). As the current is increased the initial contraction phase grows less and the secondary inhibition greater, and corresponding, apparently, to the depth of the inhibitory relaxation, the rebound becomes more and more intense (Figs. 15 B, 16 A and B).

(2) *Stretch reflexes.*

The triceps extensor preparation lends itself well to the study of stretch reflexes [Liddell and Sherrington, 1924*c*], and a few observations were incidentally made on these. In the position for recording,

Fig. 17. Stretch reflexes from taps on wrist.
A and B, "hump"; C, clonus.

with the forearm hanging downwards and thus extending the triceps, a tap applied to the wrist evokes a smart stretch reflex, comparable to the effect of a tap on the triceps tendon: the reflex is in fact a brief stretch or "tendon reflex" (Fig. 17). The height of the contraction becomes greater with increase of tone in the preparation, as after cutting the spinal cord in the upper lumbar region. Relaxation often shows a definite "hump" (Fig. 17 A and B). Sometimes the triceps exhibits an almost constant clonus, which becomes very pronounced after the induction of the stretch reflex (Fig. 17 C).

(3) *Proprioceptive reflexes.*

The triceps contracts in response to forced flexion of the opposite foreleg in a reflex similar to Philippson's [1905] in the hindlimb (Fig. 18 A); a like response may be evoked by squeezing, between finger and thumb, the opposite triceps muscle (Fig. 18 B) or its tendon (Fig. 18 C). On the contrary a squeeze of the opposite flexor group of muscles has no such effect.

Belonging in the category of proprioceptive reflexes mention may be made of a Magnus and de Kleijn [1912] reflex evocable by dorsiflexion of the neck and illustrated by contraction of the triceps extensor (Fig. 19).

Fig. 18.

Fig. 19.

Fig. 18. A, Philippson's reflex; B, squeeze of contralateral triceps muscle; C, squeeze of contralateral triceps tendon.

Fig. 19. Magnus and de Kleijn reflex from dorsiflexion of neck.

Discussion.

(1) *Actions of afferent nerves.*

Medial cutaneous nerve. This nerve being distributed only to the skin, its reflex effects are attributable solely to the stimulation of cutaneous afferents. Since there are in the medial cutaneous nerve (sympathectomized) of the cat approximately three to four times as many fine unmyelinated as myelinated fibres [Ranson and Davenport, 1931] it is logical to assume that the effects yielded by this nerve are largely due to stimulation of the fine, unmyelinated fibres; these fibres must be regarded as subserving impulses of pain. The belief, widely held to-day, that pain impulses are conveyed by fine, unmyelinated afferents is

founded on the studies of Ranson and his associates [Ranson, 1911, 1931]: it was discovered that these fine fibres, for the most part unmyelinated in spinal nerves, are the peripheral processes of small neurones in the dorsal root ganglia; the central processes enter the spinal cord through Lissauer's tract, which latter was proved experimentally to convey the afferent impulses in pain reflexes [Ranson and Billingsley, 1916]. Fine fibres corresponding to those mentioned were designated the "C" fibres by Erlanger and Gasser [1930], being found by them to have the highest threshold and to conduct at the slowest speed. Although there is considerable evidence that pain impulses may also be conducted by larger and faster fibres [Adrian, 1932], it is sufficient for our purpose to recognize that the fine unmyelinated fibres play an important rôle in pain conduction. It is these fine fibres then which are mainly involved as afferents in the reflexes from the medial cutaneous nerve.

Under the conditions of recording, with strong tone in triceps, medial cutaneous yields to both electrical and mechanical stimulation, greater positive effects than the median or ulnar nerves; further, a reversal to inhibition by strong currents is not ordinarily obtainable, as in the case of the other nerves. But in spite of the predominantly positive character of the reflex it is clear that an inhibitory component is present and indeed this forms the principal response when direct observation is used. Furthermore, given moderate tone, inhibition may be manifested by a drop in a crossed extensor background (Fig. 6 A), as well as by a gradual decline of a plateau (Fig. 5 A) or by clonus waves (Fig. 5 B) in the plateau of the positive response. Again the positive rebound shown in Fig. 4 suggests a considerable inhibitory element during the actual period of stimulation.

We may consider first the more usual reactions in which weak and strong stimuli act in the same direction, both yielding reflex contractions of triceps, the contraction size increasing uniformly with the growth of the stimulus. With reference first to the strong stimulus it is clear that all afferent fibres, large and small, must come under the influence of the intense currents used; thus, all the unmyelinated pain fibres must be evoking their reflex response. Now, since this response is contraction, or at least mainly so, and since the pain fibres are the most numerous in the nerve trunk, we must conclude that contraction is the normal response to stimulation of the pain fibres of the medial cutaneous.

Passing next to the weaker stimulus, which likewise evokes contraction, less in extent but apparently similar in nature to that from the

stronger stimulus, there is no obvious reason to assume that it is produced through the agency of any other than the pain fibres, in this case responding also to currents of less intensity. It may be that the larger afferent tactile fibres contribute, in a positive or negative way, to the responses just considered, but we cannot be sure of this, since we know nothing of their reflex potentialities.

It is of interest to compare the positive reflex effects in triceps from medial cutaneous with those observed by Graham Brown [1912] in gastrocnemius on stimulation of the ipsilateral long saphenous nerve; the resemblance rests on a similarity in anatomical distribution of both nerves and suggests a similar functional meaning of the respective reflexes.

Under the other experimental conditions referred to above medial cutaneous yields reflex inhibition of triceps. Although this response is the reverse of that just considered, there must again be stimulation of the pain fibres, since these cannot conceivably escape the influence of the strong currents used. We must, therefore, conclude that the motoneurones of triceps may, under some conditions, be excited, under others inhibited, by impulses through the same afferent pain fibres: favouring excitation would appear to be a highly active state of the motoneurones, as exemplified by powerful tone of triceps: favouring inhibition would be a somewhat depressed state of the same motoneurones; in accordance with the discussion to follow the first condition would correspond to more "central excitatory state" (*c.e.s.*) [Eccles and Sherrington, 1930], the second to more "central inhibitory state" (*c.i.s.*) [Eccles and Sherrington, 1932].

Superficial radial nerve. Respecting this nerve we have no information as to the proportion of unmyelinated and myelinated fibres, as in the case of medial cutaneous; however, since it is a cutaneous nerve, somewhat like medial cutaneous in distribution, we may reasonably assume in it a preponderance of fine, unmyelinated pain fibres. There is close resemblance between superficial radial and medial cutaneous in reflex behaviour, both yielding excitatory or inhibitory effects under appropriate conditions; the particular reaction in any case probably corresponds more or less closely to an element of normal behaviour. The various reactions may logically be considered to be due largely to stimulation of pain afferents, the outcome depending to a great extent on the initial state of the triceps motoneurones or to the amount of *c.e.s.* or *c.i.s.* present.

Dorsal interosseous nerve. This nerve is distributed to the supinator and extensor muscles of the forearm, probably also to the carpal joints,

the latter statement being based on human anatomy; regarded as a nerve of muscular supply the probable composition of dorsal interosseous may be inferred from the studies of Ranson and Davenport [1931] on the nerve to the vastus medialis in the cat: they found in this nerve, after elimination of its sympathetic components, many more myelinated than unmyelinated fibres, a condition the reverse of that in cutaneous nerves; the unmyelinated fibres, relatively few in number, probably carry pain impulses from the perimysial connective tissue [Hinsey, 1927]; they would thus correspond to those from the "C" type endings of Matthews [1933]. Apart from the motor fibres, the majority of the myelinated (afferent) fibres would be proprioceptives from the muscle spindles, since these form almost two-thirds of the total afferents from muscles [Creed *et al.* 1932].

As already stated, dorsal interosseous revealed itself, in the few cases studied, as only inhibitory of triceps. From the above considerations it may be concluded that the response is effected mainly by stimulation of the large muscle spindle afferents. In seeking for the functional counterpart of the inhibition one thinks naturally of the "negative supporting reaction" of Schoen [1926*a* and *b*]: here passive palmar flexion of the digits, with consequent stimulation of the proprioceptors of the forearm and digital extensor muscles, evokes relaxation of the triceps; faradization of dorsal interosseous, with supposedly stimulation mainly of the spindle afferents, yields the reflex underlying the reaction of Schoen. The possibility that pain afferents in dorsal interosseous also come under the influence of the current must be admitted; they may indeed contribute a modicum of excitation or inhibition to the main response from the proprioceptives; evidence on this point is, however, lacking, and it must be remarked that here, as in other cases, the reflex preparation shows itself a less perfect indicator of neural events than the various forms of oscillograph [Gasser and Erlanger, 1922; Matthews, 1928]. Broadly speaking, then, it appears that the effect from the bared afferent nerve corresponds to the negative supporting reaction.

Median and ulnar nerves. One of the principal objects of the present investigation was to determine the conditions leading to the reversal of reflex excitation of triceps into reflex inhibition. Since the reversal is most clearly obtained through the median and ulnar nerves the reflexes of these nerves will be considered in detail. The reversal is observed most readily in the state of heightened tonus following division of the spinal cord in the upper lumbar region in an animal previously decerebrated;

in these circumstances a weak stimulus applied to the afferent nerve yields contraction, whilst progressive intensification of the stimulus leads to the appearance of inhibition and rebound in increasing amounts, together with gradual extinction of the primary contraction phase (Figs. 14–16). These results are very similar to those obtained by Sherrington and Sowton [1911] in the vasto-crureus and illustrated in Fig. 3 of their paper. In explanation of the contrary influences of weak and strong currents they invoke the hypothesis of selective stimulation of groups of functionally opposed afferent nerve fibres. It becomes a matter of interest to apply their theory to the reflex reversal in triceps, particularly in the light of the more recent work dealing with the constituent fibres of nerve trunks and their electrical responses.

Beginning with the reflex contraction evocable in triceps by the weakest currents we may consider it due to stimulation of the fibres with the lowest threshold: these would correspond to the "A" group of Erlanger and Gasser [1930]; the group is composed of the largest fibres, excitable by the weakest currents, and includes afferents proved to enter the cord by the dorsal roots. Now, among the large fibres there are, of course, touch afferents, and it is possible that their stimulation evokes a response in triceps, but of this we cannot be sure. Turning, therefore, to the proprioceptive fibres we know that those coming from the muscle spindles are myelinated and of large calibre [Sherrington, 1894]; they should, therefore, be susceptible to stimulation by the weakest currents.

Considering now the possible functional meaning of the reflex contraction of triceps, presumably evoked through the proprioceptives from muscle, one naturally thinks of the "positive supporting reaction" of Schoen: in this, dorsiflexion of the digits, with consequent stretching of the forearm and digital flexors, yields rigid extension or standing in the limb [Schoen, 1926*a*], accompanied by contraction of triceps [Schoen, 1926*b*]; the proprioceptive impulses involved would be carried from the digital and forearm flexors by the median and ulnar nerves. It thus seems legitimate to conclude that the reflex contraction of triceps, evocable by a weak current stimulating presumably the large proprioceptive afferents of these nerves, is equivalent to standing or the positive supporting reaction.

Passing next to a consideration of the second phase in the response, namely the inhibitory relaxation of triceps evoked by strong currents, one may properly regard it as the normal kind of participation of triceps in a flexor or withdrawal reflex of the limb in response to painful or

nociceptive stimulation; it must, therefore, be attributed to stimulation of the high-threshold, small, unmyelinated pain fibres of the nerve; the considerations as to the pain function of these fibres mentioned under the medial cutaneous nerve apply in the case of the median and ulnar nerves.

Whilst the above conclusions are probably valid it must be admitted that a strict limitation of the current to any one group of fibres is physically impossible; thus the positive or negative response of triceps represents an algebraic sum of *c.e.s.* and *c.i.s.*, aroused through afferents of opposed influences; in the positive response there would be excess of *c.e.s.* from the large, proprioceptive afferents; in the inhibitory response there would be excess of *c.i.s.* from the small-fibred pain afferents. On the current membrane potential theory the *c.e.s.* would depend on a depolarization of the synaptic membranes involved in the reflex [Creed *et al.* 1932; Evans, 1933; Hill, 1932]. A mixture of effects like that suggested is shown in Fig. 13, in which a small contraction precedes the inhibition from the ulnar nerve. The amount of inhibition demonstrable is determined partly by the state of the muscle; thus, much more inhibition is evocable after the "shortening" than after the "lengthening reaction" (Fig. 12 A and B). The central inhibitory state is conceived by Sherrington [1930] as possibly depending on stabilization of a surface film; presumably this may be interpreted as the establishment of polarization at the synapses of the motoneurones.

The occurrence of rebound, particularly after profound inhibition, was mentioned earlier. It appears in these observations to be closely proportional to the amount of precurrent inhibition, of which it seems to be the direct counterpart; in other cases it may occur apart from inhibitory relaxation [Creed *et al.* 1932]; though electrically similar to rebound, after-discharge appears in my experiments to be a separate process, being the direct continuation of the stimulation phase of contraction. On the view that rebound is due to the persistence of the influence of afferent fibre stimulation [Creed *et al.* 1932] the afferents would be the large proprioceptive fibres. Definite rebound may, however, follow the inhibition from stimulation of the cerebellar cortex or its nuclei. This inhibition is apparently "pure," that is unmixed with any excitatory component, since it is not "reversed" by strychnine [Bremer, 1925; Miller, 1926]. There must, thus, be cases in which rebound is an actual recoil from the centre itself. The circumstance that rebound in the present experiments appears proportional in magnitude to the antecedent inhibition suggests that here a central recoil factor enters into its production, somewhat similarly to the case of cerebellar rebound.

Now the central inhibitory state was conceived as being due to polarization at synapses of the motoneurones; it would, therefore, seem legitimate to envisage rebound, in some cases at least, as an expression of powerful depolarization at the same synapses.

In the discussion thus far an attempt has been made to explain variations in the reflex responses on the basis of selective stimulation by the currents of functionally different groups of nerve fibres: this analysing action of the currents would obviously be most effective when they are approximately of threshold value for the most excitable fibres. With strong currents all afferents must be stimulated and the consequent responses would partake of the nature of algebraic summations, though contraction, inhibition and rebound would at times be partially separated through temporal dispersion. But here another possibility is encountered: Adrian and Zotterman [1926] and Matthews [1931] have shown that the rate of response in a given nerve fibre augments steadily with increase of the stimulus, being, in the case of the muscle spindle, roughly proportional to the logarithm of the load. Thus, it is quite conceivable that some of the reflex variations observed in my experiments may depend on changing frequencies of discharge in an individual fibre group. A view based on considerations of this kind has already been proposed by Adrian [1932], as a manner in which the quality and intensity of sensations may be modified.

Considerable attention has been devoted to the reversal from reflex excitation to inhibition, induced by increasing the stimulus to the median or ulnar nerve, when the triceps manifests powerful tone; this latter state ensues, as already stated, on division of the spinal cord in the upper lumbar region, following an earlier decerebration; it is attributed by Ruch [1932] to the elimination of inhibitory influences emanating from the caudal part of the cord. This state of extreme triceps tone is itself of special interest, since when present a weak stimulation of the ipsilateral median or ulnar nerve evokes further contraction of triceps, whereas, before induction of the state, the same stimulation regularly evokes inhibition. Thus the cord section brings about a reversal from inhibition to excitation in response to a given weak afferent stimulation.

In seeking for an interpretation of this reversal one naturally turns first to the studies of Sherrington [1900], v. Uexküll [1904] and Magnus [1924] respecting the influence of initial posture on subsequent reflexes. Sherrington noted, for instance, that in the spinal animal the same stimulus, namely upward pressure on the toe-pads, induces extension, if the resting posture be one of flexion but that it induces

flexion, if the resting posture be one of extension. The law was found by Magnus to be particularly exemplified by the tail of the decerebrate cat, reflex bending always occurring towards the side of the muscular movers originally put on the stretch. Analysis showed the determining factor to be proprioceptive impulses from the stretched muscles; these, playing on the corresponding motoneurones, render them hyperexcitable to afferent stimulation.

The reversals in these experiments do not, however, conform with the law just outlined: were this so, the flexor muscle being stretched by the rigidly contracted triceps, one would expect a reflex of flexion to follow, whereas exactly the opposite happens, further extension ensuing. Rather do the triceps reflexes resemble those observed by Graham Brown [1911] in the intact or decerebrate guinea-pig: here, if the preliminary posture were one of flexion the reflex response was usually further flexion: if it were first one of extension the reflex was usually further extension. Of similar nature too is the intensifying of an ipsilateral extensor reflex when the head position favours preliminary tone of the same kind [Beritoff, 1916]. Exemplifying the same law, though in the opposite sense, are the flexor reflexes, which predominate after particular cerebellar ablations, in themselves favourable to flexor tone [Miller, 1931].

As pointed out by Graham Brown [1911], there is operative in reactions of this kind a principle differing fundamentally from the "Schaltung" of Magnus. The neuronal hyperexcitability, which reveals itself in a tendency towards further reflex excitation, may be regarded as an instance of *c.e.s.*, induced in these cases by central instead of reflex factors; thus one may imagine that inhibitory influences normally emanating from the hinder part of the spinal cord result in stabilizing or polarizing the synapses of the triceps motoneurones. On cutting off these influences by lumbar cord section the synaptic membranes become depolarized with development of *c.e.s.*, expressing itself by high triceps tone and susceptibility to further excitation through afferent nerves.

It must be added that in one instance recorded above the principle of Schaltung appears to be exemplified; thus, stimulation of the superficial radial yielded inhibition of a crossed extensor reflex, although the same stimulation had yielded contraction when applied a few seconds earlier during the resting phase of the muscle.

Whilst the theory of synaptic polarization and depolarization was applied particularly to the reflexes from the median and ulnar nerves it is clearly applicable also to the reflexes from the other afferent nerves.

(2) *Stretch reflexes.*

Reference was made to the appearance of a "hump" on the descent of the triceps stretch reflex (Fig. 17 A and B); first observed in the knee-jerk by Sherrington [1915] it was interpreted by him as a "shortening reaction"; by Ballif, Fulton and Liddell [1925] it was regarded as a "myotatic appendage" following the jerk.

With respect to the afferent side of the stretch reflex valuable data have been furnished recently by Matthews [1933], who believes that his "A_1" response may be excitatory for the reflex and concludes that it comes from the flower-spray endings of the muscle spindles.

(3) *Proprioceptive reflexes.*

A forelimb Philippson's reflex, as exemplified by triceps, was described above (Fig. 18 A); Philippson's reflex in the hindlimb is always associated with the "lengthening reaction" [Sherrington, 1909*a*] in the passively stretched vasto-crureus; this lengthening reaction, as studied by Sherrington, is a reflex inhibitory relaxation evocable by stretching the muscle itself, the stretch reflex thus being depressed; recent opinion is opposed to the lengthening reaction being a "pain" reflex [Creed *et al.* 1932]; the receptors involved in it may correspond to Matthews' [1933] "B" group, situated in the musculotendinous region and thought by him possibly to exert an inhibitory influence. Arguing from the close association of Philippson's reflex and the lengthening reaction one may suppose this "B" group responsible also for the former. Receptors of this kind in the muscle and tendon must be excitable by squeezing, which was found also to evoke Philippson's reflex (Fig. 18 B and C).

Summary.

The reflex effects of various forelimb afferent nerves were studied on the "triceps extensor preparation"; this preparation comprises the medial and lateral heads only of the triceps, the long head being excluded by nerve section; the preparation is thus purely extensor or "anti-gravity"; it is referred to for brevity simply as the "triceps." The cat was decerebrate, the spinal cord sometimes being cut in the upper lumbar region. The effects recorded were evoked from the ipsilateral nerves, sometimes playing against a contralateral nerve background.

1. Medial cutaneous, a purely cutaneous nerve, yields mainly excitation in triceps, when the tone of the latter is considerable; with weaker

tone inhibition may result; the effects are attributed to stimulation of fine, pain afferents, the "C" fibres of Erlanger and Gasser.

2. Superficial radial, also purely cutaneous, yields effects like those from medial cutaneous and likewise due to stimulation of pain afferents.

3. Dorsal interosseous, a proprioceptive nerve, yields, so far as studied, only inhibition of triceps; this is attributed to stimulation of the large muscle spindle afferents. The response is believed to be equivalent to the "negative supporting reaction" of Schoen.

4. Median and ulnar nerves usually evoke inhibition of triceps. Lumbar cord section reverses this response, which becomes excitation to the same weak stimulus; a strong stimulus yields inhibition and rebound. These results are similar to those of Sherrington and Sowton in vasto-crureus.

5. Triceps excitation from median and ulnar nerves is attributed to stimulation of the large, proprioceptive afferents from the muscle spindles; it is envisaged as equivalent to the "positive supporting reaction" of Schoen.

6. Triceps inhibition from median and ulnar nerves is considered as part of a reflex withdrawal of the limb due to stimulation of fine, pain afferents.

7. Excitation and inhibition may be regarded as manifestations respectively of *c.e.s.* and *c.i.s.*; the first would correspond to depolarization, the second to polarization of the synaptic membranes of the motoneurones. Rebound is conceived as due to depolarization in the same situations. The membrane potential theory is applicable to the responses from the various afferent nerves considered.

8. The reflex reversal from median and ulnar nerves does not conform with the law of v. Uexküll and Magnus; it illustrates rather Graham Brown's principle, according to which an initial state of flexion or extension predisposes to a reflex in the corresponding direction.

9. Stretch reflexes are readily evocable in triceps, a "hump" often appearing on the descent; interpretation is made on the basis of Matthews' recent work.

10. The triceps may exhibit a Philippson and a Magnus and de Kleijn reflex. The possible bearing of Matthews' work on the Philippson reflex is discussed.

I desire to acknowledge technical assistance from Mr F. W. Claassens, Mr T. W. Stewart and Mr W. Byles.

Note.

A number of reflexes described in the preceding paper are regarded as nociceptive, being evoked by the faradization of pain fibres in the afferent nerves; in harmony with the current views of Ranson and others these pain fibres are considered as being, at least in part, unmyelinated and of fine calibre. In this connection Prof. E. D. Adrian has kindly called my attention to the work of Heinbecker, Bishop and O'Leary [1933, 1934]; in these papers the validity of the conception as to the unmyelinated nature of pain fibres is placed in question. It is probable, however, that the reflexes alluded to in my own paper are elicited through the intermediation of pain fibres, whatever the histological nature of such fibres may ultimately prove to be.

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THE EFFECTS OF HYPOTHALAMIC STIMULATION ON GASTRIC MOTILITY.

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(*Received February* 6, 1934.)

Introduction.

McCrea, McSwiney and Stopford [1925, 1927] described the effects of vagal and splanchnic stimulation on the active ("fed") and inactive ("unfed") stomach, and were able to show that the results depended to some extent on the pressure within the stomach before stimulation was commenced. Vagal stimulation was followed in the fed and unfed stomach by an initiation of gastric contractions, preceded by a latent period varying according to the initial state of the organ. Beattie [1932*a*], during an investigation of a possible parasympathetic centre within the hypothalamus, observed that faradic stimulation of the tuber region in cats caused increased gastric peristalsis, together with a flow of watery secretion from the gastric mucosa. In further development of this work, it was decided to make use of the method of recording intragastric pressure adopted by McCrea, McSwiney and Stopford, and to compare the effects on gastric motility of stimulation of different regions of the hypothalamus. By such a procedure it was hoped to obtain graphic evidence which would prove of value in the further elucidation of the hypothalamic mechanisms.

Method.

Cats were used throughout this series of experiments. We found at an early stage in our investigations that an "open" abdomen was not only inconvenient but unnecessary. It is true that our records show respiratory excursions, but these did not obscure the salient points which we were investigating.

As McCrea, McSwiney and Stopford had used chloralose as their anæsthetic, we decided to adopt this method of anæsthesia. We can agree that this drug does not affect the sensitivity of the stomach to vagal stimulation. Dale and Feldberg [1933] also used chloralose in their investigations of the effects of vagal stimulation on stomach activity and on the production in the stomach wall of an acetylcholine like substance. We have observed, however, quantitative discrepancies between hypothalamic stimulation and vagal stimulation. These we consider are due to a depression of the vagal centre within the medulla. A like conclusion was reached by Raymond-Hamet [1928], who studied the effects of adrenaline on unanæsthetized dogs and dogs anæsthetized by chloralose. In earlier experiments we had used dial, but found that it depressed the hypothalamic centres more than chloralose; for these reasons we abandoned dial in favour of chloralose anæsthesia. It was found that an interval of several hours between the administration of the anæsthetic and the commencement of hypothalamic stimulation gave the most striking results. This may be explained by the gradual diminution of the depressor action of the chloralose on the medullary and other centres.

Faradic stimulation was used in all the experiments. Bipolar electrodes were used at first, but it was found that unipolar electrodes gave a more accurately localized stimulation, with the minimum amount of damage to surrounding tissue. Three volts were used on the primary circuit, the secondary coil being placed at 10; the number of stimuli per second was approximately 30. When a unipolar electrode was used, the diffuse electrode was placed under the skin over the sacral region of the animal. Spread of the current was avoided and checked by observations of the pupil; stimulation of the anterior hypothalamus being accompanied by a contracted pupil, posterior hypothalamic stimulation by a dilated pupil.

Both fed (active) and starved (24 hours) animals were used; but it was found that in practice the resting stomach gave more uniform and more easily interpreted records. The effects of stimulation of the hypothalamus in animals with food present in the stomach were exceedingly difficult to interpret. Blockage of the œsophageal catheter probably occurred quite frequently and in addition it was found difficult to establish a control tracing before stimulation was commenced. For these reasons we decided to carry out all our experiments on the starved animal.

Following intravenous chloralose anæsthesia, a mid-line incision was made in the neck. A tracheal catheter was tied in position and the left common carotid artery isolated. An œsophageal catheter, holding a glass

nozzle at the end, was passed into the stomach, so that the nozzle projected through the cardiac orifice. The abdomen was opened, the pylorus tied, and the abdomen closed immediately, so that the minimum exposure of the gut to the air resulted. McCrea, McSwiney and Stopford state that tying of the pylorus was not a necessary step in their technique, but we found that much more accurate and comparable results could be obtained if the amount of fluid in the stomach remained constant throughout the experiment. The animal was then placed lying on its abdomen, and the head of the animal slightly raised and fixed firmly in position. 100–150 c.c. warm saline were introduced into the stomach through the œsophageal catheter, which was then attached to a water manometer. The tracheal catheter was connected with a Marey's tambour, and blood-pressure was recorded from the left common carotid. The hypothalamus was exposed by a dorsal approach, splitting the corpus callosum in the mid-line, and the floor of the third ventricle exposed by gentle separation of the lateral walls. By such an approach a clean exposure of the anterior and posterior hypothalamus can be obtained without hæmorrhage.

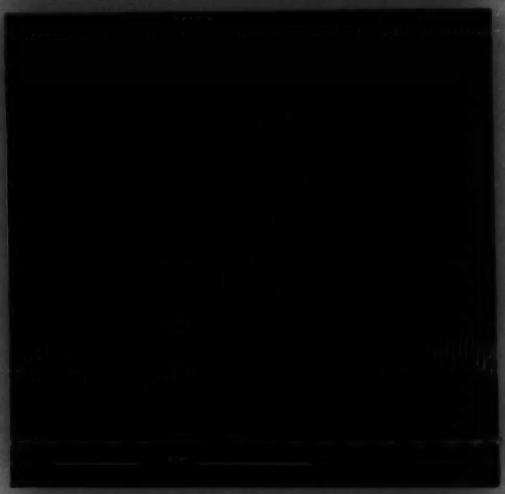

Fig. 1. Changes in the intragastric pressure following stimulation of the peripheral end of the right vagus. Left vagus sectioned. For interpretation of the figure, see text.

Discussion of results.

Stimulation of the tuber region gave constantly a rise of intragastric pressure varying from 10 to 50 mm. (Figs. 3, 4). In no case was it as striking as that produced by stimulation of the peripheral end of the cut vagus. It was observed that there was a latent period of approximately

Fig. 2. Changes in the intragastric pressure following stimulation of the peripheral end of the right vagus. Left vagus intact.

30 sec. between the commencement of stimulation and the first rise in intragastric pressure. With direct vagal stimulation the latent period was only a few seconds (never more than 10 sec.).

The rise of intragastric pressure on tuber stimulation was accompanied by a fall in blood-pressure and by a subsequent increase in peristaltic movements of the stomach (Fig. 4). Peristaltic waves were not neces-

Fig. 3. The effect of stimulation of the tuber cinereum on the intragastric pressure.

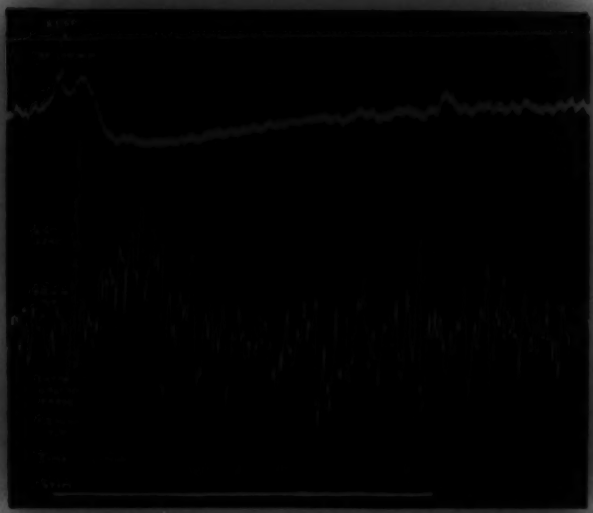

Fig. 4. The effect of stimulation of the tuber cinereum region on the intragastric pressure and the blood-pressure.

sarily coincident with an upward stroke of the intragastric pressure recording system as we have been able to confirm by direct observation of the opened abdomen. The recording system indicated only changes in the total volume and pressure of the stomach. A well-established peristaltic wave frequently caused no alteration in the level of the recording lever, as it was accompanied by a relaxation of the stomach wall proximal and distal to the site of contraction. At times a well-marked contraction

Fig. 5. Changes in the intragastric pressure and blood-pressure following mechanical stimulation (insertion of a monopolar electrode without subsequent electrical stimulation) of two regions of the anterior hypothalamus.

wave was recorded by a fall in the level of the recording lever. When the contraction waves followed one another rapidly there was invariably a rise in intragastric pressure with excursions of the recording lever which were not synchronous with the respiratory rhythm. In a completely inactive stomach only respiratory excursions were seen on the intragastric pressure tracing. When irregularities of this tracing were observed, there was no doubt that such irregularities were due to peristaltic activity as the general pressure level did not vary to any appreciable degree. This effect can be seen in Fig. 6 in the periods before and after stimulation, and in Fig. 7.

Stimulation of the tuber cinereum after unilateral vagal section showed a comparable effect to that described above, but decidedly less striking; after bilateral vagal section, the effect of tuber stimulation on both intragastric and blood-pressures was completely abolished (Fig. 7) although the stomach was still in an active state, as shown by the slight variations in the intragastric pressure tracing and by subsequent stimulation of the peripheral end of the right vagus (Fig. 8). There is therefore

Fig. 6. Tracing of the intragastric and blood-pressure changes following stimulation of the posterior hypothalamus.

experimental evidence that the efferent pathway from the anterior hypothalamus includes vagal fibres to the stomach, an efferent mechanism for which we have evidence (unpublished observations by the senior author).

Mechanical stimulation of the anterior hypothalamic region, as produced by the simple insertion of a monopolar electrode, produced a similar effect to that of electrical stimulation (Fig. 5). At the point indicated by the first arrow, the electrode was inserted anteriorly, in the neighbourhood of the supra-optic nuclear group. This resulted in a slight rise in

intragastric pressure. When the electrode was placed in the tuber region (second arrow) a much more striking result was obtained.

In the control experiments of stimulation of the vagus nerve we found, in contradistinction to McCrea, McSwiney and Stopford, that the right vagus gave a better response than the left. Further, in the resting

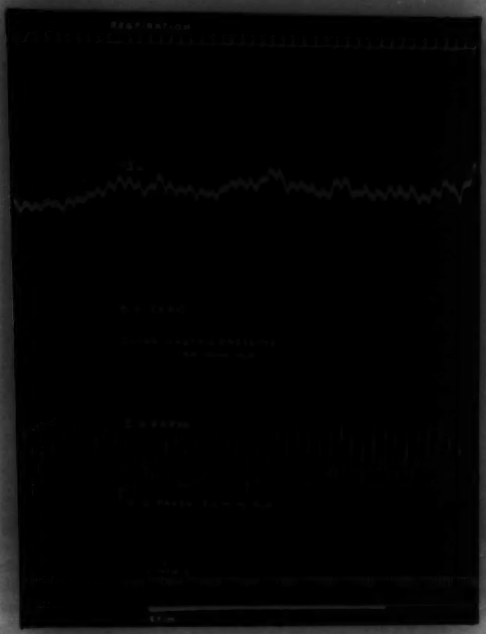

Fig. 7. The effect on the blood-pressure and intragastric pressure following stimulation of the tuber cinereum. Both vagi cut.

stomach a marked rise in intragastric pressure seemed the more significant and constant effect. Peristaltic movements were not always initiated (cf. Figs. 1, 2). On cessation of vagal stimulation the intragastric pressure fell dramatically, often below the previous level, and peristaltic activity, if obtained during stimulation, ceased or became much diminished. If vagal stimulation was continued over a long period (5 min.), the intragastric

pressure usually fell 2 or 3 min. after commencement of stimulation; a subsequent rise in intragastric pressure during the latter part of the stimulatory period frequently occurred. In the resting stomach the latent period of vagal stimulation seemed very short, often only 1 or 2 sec. This was observed by McCrea, McSwiney and Stopford, but they concluded that initiation of gastric contractions was a more constant feature of vagal stimulation than rise in intragastric pressure. In the

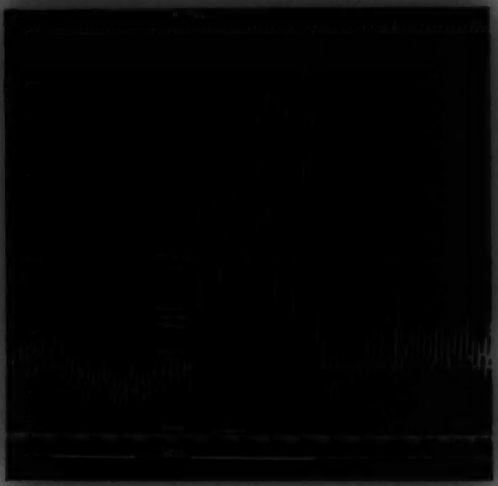

Fig. 8. The first stimulation was applied to the posterior hypothalamus, causing a fall in intragastric pressure. Both vagi were sectioned. Subsequent stimulation of the peripheral end of the right vagus produced a rise in intragastric pressure and increased peristalsis. Fig. 8 is a continuation of the same experiment illustrated in Fig. 7.

later paper McCrea and McSwiney [1927–8] found that with a low intragastric pressure (in active stomach) splanchnic stimulation resulted usually in a rise of intragastric pressure, and movements may or may not follow. With high intragastric pressures, splanchnic stimulation gave a fall in intragastric pressure with cessation of movements.

Stimulation of the posterior hypothalamus showed, in contrast to the above, a slight fall in intragastric pressure, accompanied by a rise in blood-pressure, and, what appeared still more significant, a complete

obliteration of all gastric motility. This is clearly demonstrated in Fig. 6, where only respiratory waves are seen in the intragastric record during posterior hypothalamic stimulation. More striking results were obtained when the initial intragastric pressure was high. The gastric phenomena produced by posterior hypothalamic stimulation were not affected by section of the vagi.

It would appear from our results that a well-defined similarity exists between the effects of tuber stimulation and vagal stimulation on gastric motility, and similarly between posterior hypothalamic stimulation and splanchnic stimulation, though we never obtained a rise in intragastric pressure on pure posterior hypothalamic stimulation. This similarity is more qualitative than quantitative. The difference is probably due at least in part to the depressant action of chloralose on the hypothalamic and vagal centres.

Beattie has shown [1932*b*] that there exists probably in the hypothalamus two distinct mechanisms—an anterior or "parasympathetic" and a posterior or "sympathetic"—and the results recorded above appear to give a direct experimental proof of such an arrangement, in so far as gastric motility is concerned.

Summary.

1. Faradic stimulation of the tuber region in the fasting cat, under chloralose anæsthesia, resulted in a rise of intragastric pressure, with a subsequent increase in peristaltic movements of the stomach.

2. Section of both vagi abolished this effect.

3. Faradic stimulation of the posterior hypothalamus in the fasting cat, under chloralose anæsthesia, resulted in a slight fall in intragastric pressure and a complete obliteration of all gastric motility.

We wish to acknowledge with thanks grants in aid of the expenses of the above research from the Banting Research Foundation, Toronto, and the Cooper Fund of McGill University.

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CONDUCTION IN THE CERVICAL SYMPATHETIC.

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(*Received February* 12, 1934.)

These experiments were originally undertaken to determine first, whether it was possible to make accurate quantitative measurements of the response of unstriated muscle to nerve stimulation and, second, if this were feasible, to use the muscle response for the investigation of conduction through the sympathetic ganglia. The nictitating membrane of the cat was chosen, since it affords a preparation which can be used *in situ*, with its blood supply intact and which gives a measurable response to a single volley set up in the cervical sympathetic.

Method.

Cats were anæsthetized with ether and decerebrated, and the III, VI and V cranial nerves were divided immediately afterwards. This ensures immobility of the eye, twitching of which otherwise seriously interferes with recording [Rosenblueth and Bard, 1932]. The carotid artery of the side to be used, the left, was not tied and was occluded for as short a time as possible to control bleeding. The left cervical vago-sympathetic trunk was dissected and the sympathetic alone was tied low in the neck. Considerable care was taken to retain the blood supply of the nerve, and for this reason the vagus and the sympathetic were not separated at the point of stimulation. Control experiments showed that the conjoint excitation of vagus and sympathetic did not affect the accuracy of the results. Records of the contraction of the nictitating membrane were taken with a very light isometric torsion myograph of the pattern described by Eccles and Sherrington [1930]. The optical magnification was 100×, a tension of 1 g. giving a deflection of about 1 cm. on the camera and allowing a shortening of the muscle of not more than 0·1 mm. The head was immobilized by a threaded rod bolted into the parietal bone and by a steadying bar applied to the jaw.

Head clamps are undesirable since they may cause occlusion of the facial artery. The stimuli used were the discharges of a 0·05 μF condenser through the nerve in series with 10,000 ohms, the nerve being shunted by a further 10,000 ohms. Condenser discharges are very much more satisfactory than induction shocks, since induction shocks of very high intensity, while spreading considerably to surrounding tissues, may yet fail to cause maximal excitation of the nerve. The electrodes applied to the nerve were silver wires shielded in glass tubes. The stimuli were timed by a Lucas pendulum.

Results.

1. *The preganglionic summation curve.*

(*a*) *Effect of a single stimulus.*

A single maximal volley set up in the cervical sympathetic, central to the superior cervical ganglion, results in a "twitch" (Fig. 1). The

Fig. 1. Series of isometric myograms of the nictitating membrane. The figures show the interval between stimuli. Time, 0·2 sec.

tension developed varies from animal to animal from a fraction of a gram to 2 g. The latency, with electrodes about 5 cm. from the ganglion, is of the order of 150σ. The tension developed as a result of a single preganglionic volley tends to fall off during an experiment. This falling-off in response does not occur in contractions in which greater tensions are developed as a result of more than one stimulus.

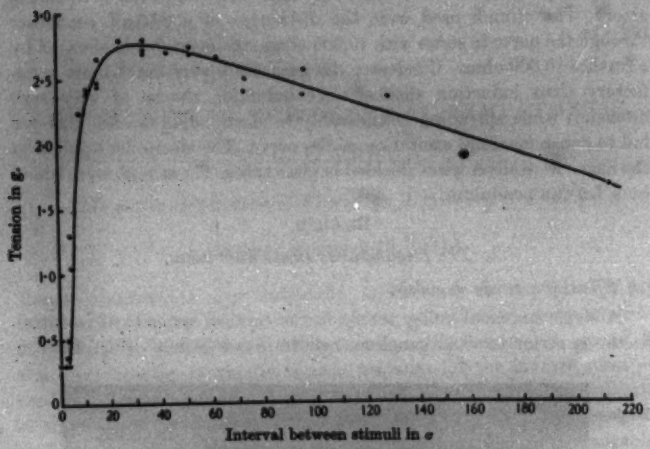

Fig. 2. Summation curve with preganglionic stimulation.

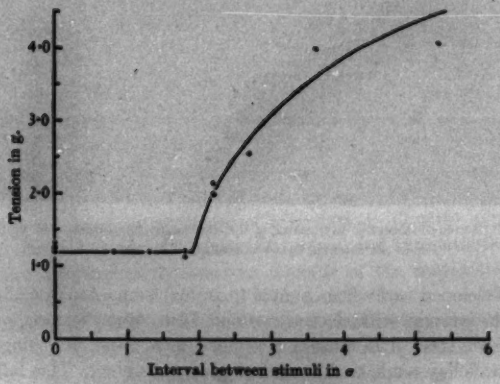

Fig. 3. Curve showing least interval for summation with preganglionic stimulation.

(*b*) *Effect of two stimuli.*

Two supra-maximal stimuli were applied to the nerve, either through single electrodes or through a pair of electrodes separated by 1 cm., the second stimulus falling in a part of the nerve traversed by the volley set up in the first pair of electrodes. A typical summation curve is shown in Fig. 2. For a period of about 2σ the tension developed is equal to that from a single volley alone, after which the curve rises steeply to reach a maximum between 20 and 40σ and thence declines so slowly that stimuli separated by as much as 500σ give a contraction which is 75 p.c. of the peak tension reached. The least interval at which muscular summation takes place, using single electrodes, varies between $1{\cdot}3$ and $2{\cdot}0\sigma$, the mean of a number of experiments being $1{\cdot}7\sigma$. When twin electrodes were used, with the cathodes 1 cm. apart, the measurement of this interval gave the following figures: longest value $3{\cdot}5\sigma$, shortest $2{\cdot}5\sigma$ with a mean of $3{\cdot}0\sigma$ (Fig. 3). It must be understood that the least interval for muscular summation does not necessarily represent the refractory period of any part of the system.

(*c*) *Discussion.*

It has been suggested by Querido [1924, 1925], on the basis of measurements of the response of the nictitating membrane to pre- and postganglionic stimulation, that the superior cervical ganglion acts as a transformer of the nerve impulses transmitted to it, widely different frequencies of incident impulses being converted in the ganglion to a frequency which is optimal for the muscle fibres which are supplied by the postganglionic nerves. He implies, in fact, that the ganglion is endowed with some of the properties of the central nervous system. Veach and Pereira [1925] and Knoefel and Davis [1933] have thrown doubt on Querido's results, using identical methods of investigation. Bishop and Heinbecker [1932] have analysed the action potentials of the cervical sympathetic supply of the eye of the cat and Belgian hare by means of the cathode ray oscillograph. It is not clear from their description to which exact group of fibres belong those supplying the nictitating membrane, but they give a refractory period of $3{\cdot}5$–$4{\cdot}5\sigma$ for the fibres and a conduction velocity of 12 m. per sec.

These figures appear to place the fibres of the nictitating membrane in the B_2 group of visceral nerves described by the same authors [Bishop and Heinbecker, 1930]. The B_2 fibres have the following properties: conduction velocity, 15–10 m. per sec.; threshold approximately ten times that of the *A* fibres, and an absolute refractory period

of 2·2–4·5σ. A similar group of fibres in the cervical sympathetic has been described by Eccles [1933]. These figures for the refractory period of the nerve agree reasonably well with the values found in the present experiments for the least interval for muscular summation, *i.e.* a mean interval of 1·7σ for both stimuli delivered through the same electrodes and a mean of 3·0σ when the electrodes are separated by 1 cm., the latter figure being compatible with a conduction velocity of 10 m. per sec.

As regards the conduction through the ganglion, Bishop and Heinbecker [1932] find that the action potentials in the postganglionic fibres resulting from the second of two volleys are only maximal when the preganglionic stimuli are separated by 20–30σ, the postganglionic potential gradually diminishing as the preganglionic stimuli are approximated. They suggest that 20σ represents the longest refractory period of the ganglionic synapses.

In our experiments there are three obvious explanations of the summation curve obtained:

(i) That the refractory period of the ganglion cells is of the same order of magnitude as that of the preganglionic fibres, *i.e.* about 2σ. The shape of the summation curve then would be determined by the summation process in the muscle.

(ii) That, as suggested by Bishop and Heinbecker, the postganglionic volley set up by the ganglion cells is reduced at intervals less than 20σ by the refractory period of some of the cells.

(iii) That there exists in the ganglion a process similar to that in the central nervous system, whereby the effects of two incident volleys, separated temporally, may be summed. The least interval for muscular summation would then be the refractory period of the preganglionic fibres.

Considering the last possibility, this implies that all the ganglion cells are not excited to the threshold of their discharge by a single volley, the subliminal fringe left over from the first volley summing with the excitatory effects produced by the second. It is apparent from the records and the summation curves that the tension produced by two volleys summing maximally, *e.g.* at 20σ, is as great as seven times that produced by a single volley, and this is in apparent agreement with the occurrence of a summation process in the ganglion. However, it has been pointed out by Billingsley and Ranson [1918] that the preganglionic fibres are considerably fewer in number than the postganglionic, there being thirty-two ganglion cells to each preganglionic fibre. This means, presumably, that each preganglionic fibre excites a number of ganglion cells,

whose axons constitute the postganglionic fibres. It follows that, if a single preganglionic volley fails to excite all the ganglion cells, then a single maximal postganglionic volley should produce a contraction considerably greater than that produced by a single preganglionic volley, provided that the preganglionic volley results in a single discharge of the ganglion cells. As will be shown later, a single postganglionic volley does not, consistently, have an effect significantly different from that of a single preganglionic volley.

As regards the other explanations, it is impossible to decide, from the preganglionic summation curve alone, what part the ganglion plays in determining the shape of the curve from 2σ onwards. Moreover, there is no evidence in such curves for the time relations of conduction through the ganglion and the nature of the impulses set up in the postganglionic fibres.

2. *The postganglionic summation curve.*

Postganglionic stimulation was effected by the application to the postganglionic fibres of electrodes, in the form of two steel hooks, shielded in a glass tube and so arranged that the hooks lay on opposite sides of the nerve. The position of the ganglion and the short length of the postganglionic fibres available necessitated the application of the electrodes with the anode not more than 2 mm. from the ganglion; the cathode lay headwards. Considerable care was taken to avoid disturbance of the ganglion and its connective tissue sheath and blood vessels.

(*a*) *Effect of a single stimulus.*

The effects of a single postganglionic volley are indistinguishable from those of a single preganglionic volley. When recording the effects of stimulation of both pre- and postganglionic fibres in the same experiment it is not unusual to observe that the tension resulting from a preganglionic stimulus is less than that from a postganglionic. This difference is, however, by no means constant and is to be attributed to the depression of some of the ganglion cells as a result of the manipulations entailed in the application of the electrodes. In one experiment, immediately after setting up the animal, preganglionic stimulation was without effect and the response returned suddenly some 4 hours later, then giving contraction values almost exactly equal to postganglionic stimulation. This appears to be compatible with the explanation advanced above for the inequalities between pre- and postganglionic stimulation.

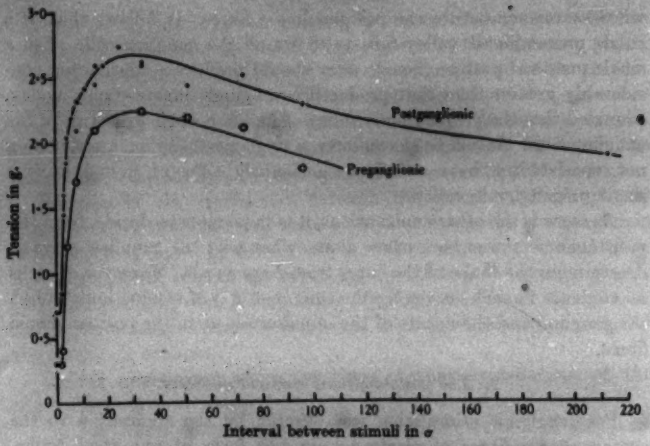

Fig. 4. Summation curve with pre- and postganglionic stimulation in the same animal.

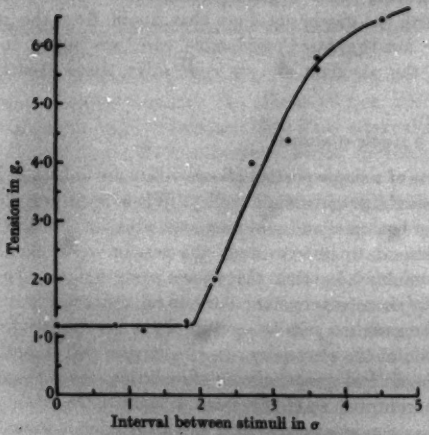

Fig. 5. Curve showing least interval for summation with postganglionic stimulation.

(*b*) *Effect of two stimuli.*

Fig. 4 shows the postganglionic summation curve, and with it the preganglionic curve obtained in the same experiment. It is clear that the two curves have identical time relations and general form, the only difference in this instance being the difference in magnitude discussed above. Fig. 5 shows the curve at short time intervals and the interval for muscular summation. The values obtained are in close agreement with those for preganglionic stimulation, viz. preganglionic $1{\cdot}3$–$2{\cdot}0\sigma$; postganglionic $1{\cdot}6$–$2{\cdot}0\sigma$.

(*c*) *Discussion.*

It appears, therefore, that the characteristic form of the summation curve is not imposed upon it by the transmission through the ganglion, but rather by the nature of the summation process in the muscle and by the properties of the postganglionic fibres. The obvious conclusion is that the refractory period of the ganglion cells is of the order of 2σ and that the diminishing efficacy of the second of two preganglionic volleys, as the interval between them becomes less than 20σ, is due to its arrival at the muscle at a time which is not optimal for the summation process.

Furthermore, the great similarity of the pre- and postganglionic summation curves is strong presumptive evidence for the simplicity of the transmission process through the ganglion, that is, that a single preganglionic stimulus gives rise to a single volley of impulses in the postganglionic fibres. Nevertheless, it is not possible to decide this point definitely from the summation curves from pre- and postganglionic stimulation alone, and no information can be obtained on the velocity at which the impulse travels through the ganglion. These problems were investigated by studying the summation between a volley set up in the preganglionic fibres and a volley in the postganglionic trunk.

3. *Summation between pre- and postganglionic volleys.*

The arrangement of the electrodes is shown in Fig. 6. *A* represents the preganglionic electrodes, *B* the postganglionic electrodes and *G* the ganglion. The distance *AG* was usually about 5 cm. and *GB* not more than 3 mm. A stimulus was applied at *A* and was followed at varying intervals by a stimulus at *B*. The summation curve obtained in one such experiment is shown in Fig. 7.

Considering the points at which *A* precedes *B* by 20σ: the volley set up at *A* will pass to the ganglion, will be transmitted there and the postganglionic impulses will travel to the muscle, to be followed by the volley

set up at *B*. The interval separating the volleys reaching the muscle will be determined by the time interval between the stimuli, less the conduction time *AG*, the conduction time in the ganglion (*G*) and the conduction time *GB*. That the summation curve obtained in these

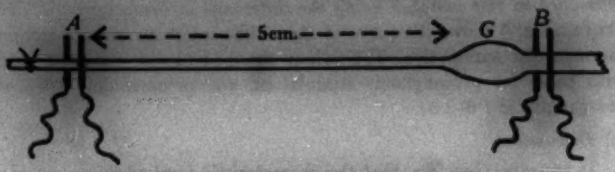

Fig. 6. Diagram of position of electrodes for simultaneous pre- and postganglionic stimulation.

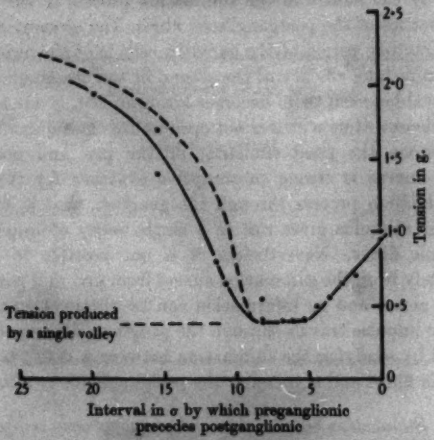

Fig. 7. Curve showing summation of volleys set up in the pre- and postganglionic fibres.

conditions does not differ materially from that resulting from the summation of two preganglionic volleys is shown by the dotted curve, which was obtained from the preganglionic fibres some 2 hours previously in the same preparation. The curve is so arranged that the two points for the least summation intervals correspond.

Between the intervals 9·0 and 5·0σ it is clear that the two volleys are interfering, since the tension developed is equal to that produced by a

single volley. The point of initial interference, *i.e.* 9·0σ approximately, enables one to determine the conduction time from the point of stimulation, through the ganglion to the postganglionic electrodes and, since interference will begin as soon as the postganglionic stimulus falls within the absolute refractory phase, following the ganglionic discharge through the postganglionic fibres, one must add to this time the refractory period of the postganglionic fibres.

In other words:

$$AG+G+GB+\text{postganglionic R.P.}=9\cdot0\sigma.$$

Assuming the conduction time in the preganglionic fibres to be 10 m. per sec. and neglecting GB, since the distance is only a matter of 3 mm., and allowing 2σ for the refractory period of the postganglionic fibres, then

$$G=9-5-2\sigma$$
$$=2\sigma.$$

In experiments of this type the longest time to the point of initial interference was 10σ and the shortest $4\cdot5\sigma$. The difficulty in making accurate determinations lies in the fact that strong stimuli are necessary and spread of stimulating current from the preganglionic electrodes is inevitable. In an experiment in which the preganglionic stimulus was reduced to a submaximal intensity, the interference interval was found to be 6σ. It appears, therefore, that assuming a conduction velocity of 10–15 m. per sec. the delay in the ganglion may be as long as 2σ, but is frequently much less.

In the curve shown the period of interference lasts for 4σ and the obliteration of the effects of one volley is complete. The first 2σ of this period, that is, after the point of initial interference, must be due to the fact that the ganglionic discharge, passing into the postganglionic fibres, renders them refractory and so the stimulus at B is ineffective. After this period has elapsed, the stimulus at B becomes effective and sets up (*a*) a volley which passes peripherally, and (*b*) a volley which is antidromic in direction. This antidromic volley will render the fibres BG refractory to any impulses discharged by the ganglion for a further 2σ, quite apart from any effect which the antidromic volley may have upon the ganglion cells. Thus it is possible to account for the absence of summation during the period 9·0–5·0σ almost entirely on the basis of the refractory period of the postganglionic fibres.

The completeness of the interference between the pre- and postganglionic volleys in this instance suggests strongly that the ganglionic discharge is single and synchronous or at least, if there is any temporal

dispersion of the impulses, it is over a period of less than 2σ. In some experiments the tension developed during the period of interference was greater than that resulting from a single volley. Provided that the postganglionic volley was maximal, the only feasible explanation of such an occurrence is the excitation more than once of some of the postganglionic fibres. In other words, either the ganglion cells discharge more than once or their discharge is so asynchronous that some impulses enter the postganglionic fibres more than $2{\cdot}0\sigma$ after the first discharge. Repetitive discharge of the ganglion cells is unlikely and it is significant that, in those experiments in which the interference was incomplete, the tension resulting from a preganglionic volley was less than that from a postganglionic, suggesting that the ganglion was depressed.

It appears, then, that the preganglionic stimulus evokes from the ganglion a single volley in the postganglionic fibres, after a delay which is certainly less than 8σ and which may be as short as 1σ in certain conditions. The evidence on the whole points to a delay of about 2σ. This value is in close agreement with the values obtained by Eccles [1933] when recording the action potentials developed in the ganglion itself during preganglionic stimulation. The volley in the postganglionic fibres is probably dispersed, in natural conditions, over less than 2σ, but damage to the ganglion may cause the dispersion to be greater.

4. Effect of antidromic impulses on the ganglion.

It is clear from Fig. 7 that, during the period 0–5σ interval between the volleys, the impulses set up in the postganglionic fibres have come from cells which have been exposed to the effects of an antidromic volley. Fig. 8 shows the continuation of such a curve, with points obtained at longer intervals after the setting-up of the antidromic impulses. If the antidromic volley had no effect on the ganglion, it is evident that the curve obtained should be symmetrical about an axis drawn midway through the period of interference. This is manifestly not so in the curve of Fig. 8. It appears, then, that the antidromic volley set up in the postganglionic fibres produces depression of the conduction through the ganglion, which may last as long as 15σ. There are, however, two other possible explanations of the depression of the summation curve. First, it is conceivable that, on account of the strong stimuli necessary to excite the postganglionic fibres maximally, there is escape of the stimulus over the ganglion and consequent blockage, in part, of the volley ascending in the preganglionic fibres. That such escape does occur in certain circumstances can be shown, but the resulting curve is distinctive.

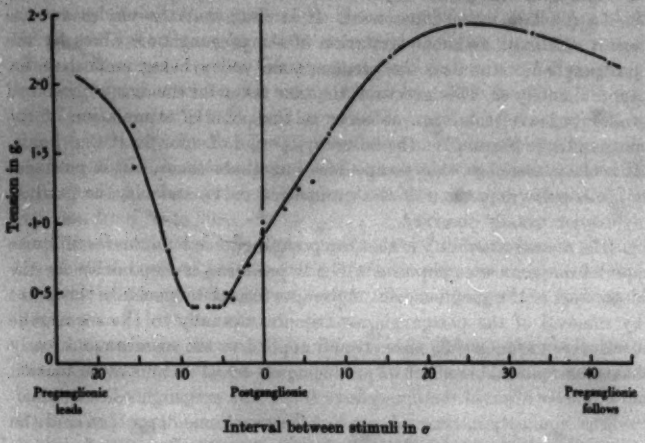

Fig. 8. Summation curve showing depression of the ganglion by antidromic impulses.

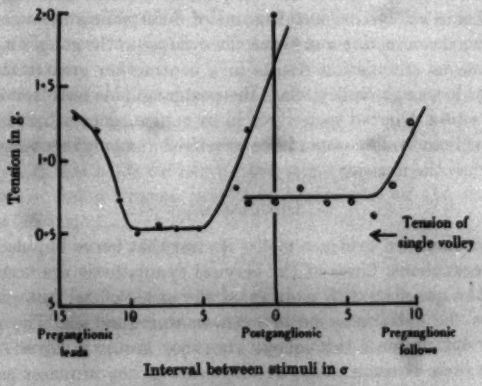

Fig. 9. Summation curve showing effects produced by the spread of the postganglionic stimulus to the preganglionic fibres.

In Fig. 9 the points (•) were obtained half an hour before the circles (○), late in an experiment. It is clear that the circles are the result of almost maximal excitation of the preganglionic fibres by the postganglionic stimulus, the preganglionic volley being ineffective for approximately 6σ. This interval is the time taken for the escape-produced volley to travel the 5 cm. of nerve to the point of stimulation of the preganglionic fibres, plus the refractory period of some 2σ at that point. It is clear, therefore, that escape does sometimes occur, but it produces a rectangular depression of the summation curve and not the gradual depression usually observed.

The second possibility is that the postganglionic stimulus itself, quite apart from the nerve impulses which it produces, is responsible for the depression of the ganglion cells. Attempts further to elucidate this point by removal of the postganglionic stimulus actually to the membrane itself were unsuccessful, since stimuli applied to the membrane directly have the remarkable effect of producing an equal increment in tension at whatever interval they precede or follow the preganglionic stimulus.

The similarity in time relations of the ganglionic depression and the depression of the reflex activity of the spinal cord as a result of an antidromic volley in the motor nerve [Eccles and Sherrington, 1931; Eccles, 1931] would suggest that the depression in these experiments is genuine, and not due to spread of the local effect of the stimulus.

It is interesting to note that the use of a simultaneous pre- and postganglionic volley is a useful means of determining the existence of unidirectional conduction and hence of a synapse in the ganglion. If such a simultaneous stimulation results in a contraction greater than that produced by a single volley, then the postganglionic antidromic volley must have been blocked somewhere in its course, since otherwise it and the preganglionic volley would have interfered and only one volley would have reached the muscle.

Discussion.

The accumulated evidence makes it clear that nerve impulses set up in the preganglionic fibres of the cervical sympathetic are transmitted through the ganglion with a delay of the order of 2σ, but in certain conditions the delay may be of even shorter duration. The delay is probably not uniform throughout the fibre group involved and accordingly there is some temporal dispersion of the impulses set up in the postganglionic fibres. The least interval for muscular summation (2σ) appears to approximate closely to the refractory period of the

ganglion cells, although it is probable that the shortness of the ganglionic refractory period is exaggerated in these experiments by the fact that the second volley, travelling as it does in the relative refractory period of its predecessor, reaches the ganglion later than would be suggested by the interval at which it is set up. The value of 20σ found by Bishop and Heinbecker [1932] for the refractory period of the ganglion is clearly too long. Eccles [1933] attributes the discrepancy to anæmia of the ganglion in Bishop and Heinbecker's experiments, since he finds that occlusion of the carotid lengthens the refractory period of the ganglion from 3σ to 20σ.

There is no evidence in these experiments that transmission through the ganglion involves processes allied to facilitation and summation, such as occur in the central nervous system. In this respect the findings of Bishop and Heinbecker are confirmed.

As regards the nature of the transmission process, the shortness of the refractory period and the rapidity with which the impulse passes through the ganglion are noteworthy. Recently, Feldberg and Gaddum [1933] have advanced evidence that acetylcholine is involved in the passage of the nervous impulse through the superior cervical ganglion. If this is true, then the shortness of the refractory period and the absence of summation and facilitation point to the very rapid removal of the transmitter after its action is over. It is probable that further investigation of the ganglion under more severe bombardment with preganglionic impulses will reveal peculiarities of the transmission process which are not apparent when no more than two successive volleys are transmitted.

Matthews [1931] has suggested that the phenomena of conduction, as ordinarily investigated by two volleys, may not give a true picture of the happenings in nerve which is conducting a series of rapidly following impulses. If this holds for nerve, then it is even more likely to be true for a transmission process involving a structure of the nature of a synaptic junction.

Summary.

1. Optical isometric myograms of the contraction of the nictitating membrane of the decerebrate cat have been recorded.

2. A single maximal volley set up in either the preganglionic or the postganglionic fibres of the cervical sympathetic results in a contraction of the nictitating membrane. Two volleys evoke a greater contraction, provided that the interval between them is sufficiently long.

3. The least interval for muscular summation was found in the preganglionic fibres to be between 1·3 and 2·0σ, and in the postganglionic fibres to be between 1·6 and 2·0σ.

4. Summation curves were constructed relating interval between stimuli to tension developed. The close similarity between the preganglionic and postganglionic summation curves suggests that the transmission process through the ganglion is simple, a single preganglionic volley giving rise to a single volley in the postganglionic fibres.

5. Evidence is advanced suggesting that the superior cervical ganglion has a refractory period of the order of 2σ. There is no evidence in these experiments for the occurrence of summation, facilitation or after-discharge in the ganglion.

6. The time relations of conduction through the ganglion were investigated by study of the summation of the effects of a volley set up in the preganglionic fibres with those of a volley set up in the postganglionic fibres.

7. It was found that the impulse is transmitted through the ganglion with a delay which may be as long as 2σ, but which is frequently much less.

8. The impulses set up in the postganglionic fibres, as a result of a preganglionic volley, are dispersed temporally over less than 2σ, but damage to the ganglion may increase this dispersion.

9. Antidromic impulses entering the ganglion produce a depression of the ganglion lasting some 15σ.

The expenses of this investigation have been defrayed in part by a grant from the Government Grants Committee of the Royal Society.

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THE REPETITION OF CERTAIN EXPERIMENTS ON WHICH MOLITOR AND PICK BASE THEIR WATER-CENTRE HYPOTHESIS, AND THE EFFECT OF AFFERENT NERVE STIMULI ON WATER DIURESIS.

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(*Received February* 26, 1934.)

The complexity and the fundamental nature of the issues involved in the regulation of the water content of the body and its partition have convinced most workers of the need for postulating some central control, but a wide divergence of opinion obtains as to its nature. Molitor and Pick have advanced the hypothesis that the control is vested in a centre situated in the neighbourhood of other vegetative centres in the mid-brain, which when stimulated by a fall in the water content of the blood, causes the tissues to liberate water into the blood. On the other hand, an excess of water in the blood is dealt with by the kidneys. They are not, however, decided as to whether the water centre effects its control by nervous or hormonal means. Like the other vegetative centres the water centre is under the inhibiting control of the cerebral cortex, and if this restraining influence is removed either surgically or by optimum doses of such narcotics as paraldehyde or chloralose, the centre becomes over-active and water diuresis is no longer inhibited by posterior pituitary extract. This extract, in their view, has no direct action on the kidneys or the renal circulation, but acts, either directly or through the water centre, on the tissues, enabling them to hold more water.

This hypothesis is based almost entirely on experiments devised to investigate the anti-diuretic action of posterior pituitary extract. Molitor and Pick [1924] found that the inhibition of water diuresis caused by this extract could not be overcome either by vaso-dilator substances such as amyl nitrite, sodium nitrite, nitroglycerine or papaverine or by diuretics such as caffeine, theobromine or theocin. On the

other hand, it could be overcome by the administration of such diuretics as sodium chloride, glucose and, most powerful of all, urea, which act in virtue of the osmotic pressure they exert. In 1925 these authors stated that posterior pituitary extract did not inhibit water diuresis either in rabbits under paraldehyde narcosis or in those which had been decerebrated, the tests being carried out two or three days after the animals had recovered from the operation. In 1926 they further affirmed that: (*a*) the injection of posterior pituitary extract into dogs under a suitable degree of chloralose narcosis has no influence on the course of water diuresis; (*b*) if the extract be injected intrathecally it inhibits water diuresis even when amounts are used which have no effect when introduced intravenously; (*c*) the inhibition of water diuresis persists for a longer time when the extract is injected intrathecally than when injected subcutaneously.

These conclusions, if they can be substantiated, offer strong evidence in favour of the water-centre hypothesis and indisputable evidence (notwithstanding the fact that Starling and Verney [1925] reported that posterior pituitary extract lowered the rate of secretion of urine in the heart-lung-kidney preparation) that posterior pituitary extract does not exert its anti-diuretic activity directly on the kidneys or the renal circulation. For these reasons Prof. E. B. Verney suggested that it was desirable that the experiments reported by Molitor and Pick in 1926 should be repeated. Before these experiments are described reference will be made to a paper published by Janssen in 1928. Working with rabbits he found: (1) that division of the spinal cord at the level of the fifth cervical vertebra, together with section of both vagi, did not interfere either with the normal concentration of urine or with water diuresis, which was inhibited in the ordinary manner by the injection of posterior pituitary extract; (2) that a given amount of posterior pituitary extract inhibited water diuresis as effectually when injected subcutaneously as when injected intrathecally; (3) that posterior pituitary extract inhibited water diuresis in decerebrate animals, although the tuber cinereum, infundibulum, thalamus, and the brain stem down to the corpora quadragemina had been removed. These experiments, if confirmed, would prove that the water centre could not exert its control by nervous impulses and that posterior pituitary extract could not affect the tissues through the mediation of the water centre. They would not, however, invalidate the hypothesis advanced by Molitor and Pick, which affirmed the possibility that the water centre effected its control by hormonal means and that the posterior pituitary extract acted directly on the tissues.

Experimental work.

The experiments were performed on young bitches on which a plastic operation exposing the urethra [see Klisiecki, Pickford, Rothschild and Verney, 1933] had been performed. A preliminary hydrating dose of 250 c.c. of tepid water was given through a stomach tube and the animal returned to its cage. Some two hours later it was placed in a Pavlov's stand and a female rubber catheter was passed and fixed in position. The urine was collected in a graduated glass cylinder and the amounts were read at 10 min. intervals. After the urine flow had returned to its resting rate a further 250 c.c. of water were introduced into the stomach in the same manner. The time occupied in administering the water was 1½ min.

The effect of the intravenous injection of posterior pituitary extract on water diuresis in dogs under chloralose narcosis.

It is postulated by Molitor and Pick [1925*b*] that the water centre normally receives inhibitory impulses from the cerebrum and that these may be inhibited by a suitable dose of chloralose which selectively narcotizes this area of the brain. It follows that a dog under chloralose narcosis should excrete more urine in a given period after water has been administered than when normal.

Chloralose dissolved in 250 c.c. of water was administered through the stomach tube to three different bitches on thirteen occasions. The optimum dose of this drug was found to be 0·125 g. per kg. of body weight. When this amount was given the animal became sleepy, sagged in the middle, and without passing through any stage of excitement fell fast asleep, usually in about 30 min. As the drug became effective the animal had to be supported by bands passed round the chest and abdomen and fixed to the beam of the stand. The head had also to be supported in a similar manner. While "asleep" it did not mind the prick of a needle or being pinched, but exhibited movements in response to a sharp noise or to its ears being flicked. The renal response to the ingestion of water was usually good, but except in the case of one bitch the amount of urine secreted in a given period and the maximum rate of secretion were lower than normal. If a smaller dose of chloralose was given the animal passed through a stage of intense excitement and water diuresis did not occur. Moreover, possibly because of the violent movements, hæmaturia occurred and blood was noticed in the fæces, which were somewhat violently expelled (Fig. 1).

The preparation of posterior pituitary extract used was "Infundin" (Messrs Burroughs and Wellcome) the oxytocic content of which is standardized in international units. All the ampoules used were taken from one batch which was kept in cold storage. Five units (0·5 c.c.) were diluted with physiological saline to make 10 c.c., and of this 1 c.c. was taken and added to 9 c.c. of physiological saline. Successive dilutions were made in like manner until the required dose was obtained. The standard amount of fluid injected was 1 c.c., this was introduced into the lateral metatarsal vein while the animal stood in the stand, and with the minimum of disturbance. The smallest amount of "Infundin" which

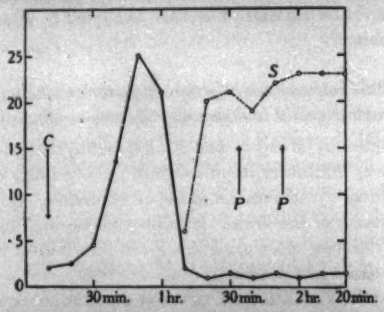

Fig. 1. Curve showing the effect of an insufficient dose of chloralose on water diuresis. At *C* 0·075 g. per kg. of chloralose, dissolved in 250 c.c. of water, was given through a stomach tube. Water diuresis commenced in the normal manner, but at the end of 40 min. the animal became very excited and remained in an excited state during the rest of the experiment. As the rate of urinary secretion fell excessive salivation occurred, the amounts collected being recorded in curve *S*. "Infundin" 0·05 unit was injected subcutaneously on two occasions at points *P* and was without effect on the rate of salivary secretion. The urine secreted during this period contained albumin and blood, blood being also present in the fæces. In this and in the subsequent figures water was given at the points the curves commence; the ordinates represent the amounts of urine or saliva secreted in the preceding 10 minute interval and recorded in c.c.; and the abscissæ represent the times in 30 minute intervals.

when injected intravenously caused "minimal inhibition" of water diuresis was determined for each of the three bitches used, and the amount required was remarkably constant for each animal. By "minimal inhibition" is meant the falling of the rate of urinary secretion during water diuresis from near the peak to approximately the resting rate for about 10 min., after which time it again rises to reach a maximum some

40 min. later. No determinations were made, either in the normal or the narcotized animal, without a previous administration of 250 c.c. of water to hydrate the tissues.

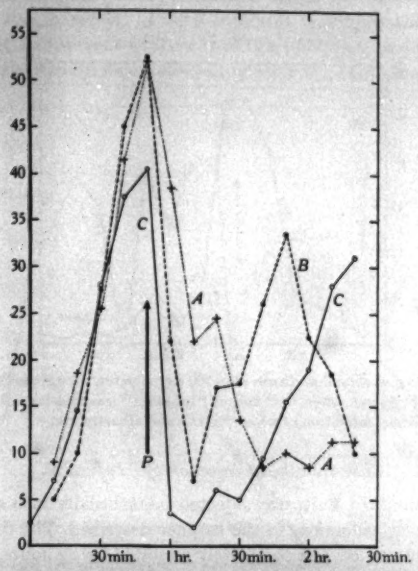

Fig. 2. These three curves were obtained with the same bitch. Curves *A* and *C* represent the effects on water diuresis caused by the intravenous injection of 0·0005 unit of "Infundin" at *P*, the animal being normal in curve *A* and under chloralose narcosis in curve *C*. It will be observed from curve *A* that the animal secreted 85 c.c. of urine during the 30 min. following the injection, while in curve *C*, during the corresponding period of time only 11·5 c.c. were secreted. It is therefore evident that the same amount of "Infundin" inhibited water diuresis more effectively when the animal was under chloralose narcosis than when normal. Curve *B* shows the effect on water diuresis of the intrathecal injection of 0·005 unit of "Infundin," the animal being normal. It is clear that an amount of "Infundin," injected intrathecally into the normal animal inhibited water diuresis less effectively than one-tenth of the amount injected intravenously into the animal under chloralose narcosis.

So soon as the animal was fast asleep and the peak of water diuresis had occurred, the smallest amount of "Infundin" which caused minimal inhibition of water diuresis in the normal state was injected intra-

venously. On each and every occasion the same amount of "Infundin" inhibited water diuresis just as effectually as when the animal was normal. Indeed in one bitch the intravenous injection of 0·0005 unit of "Infundin" was more effective when the animal was narcotized than when normal (Fig. 2, see also Fig. 3).

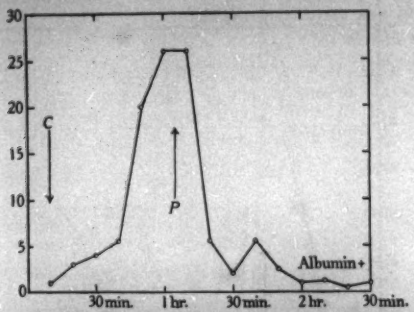

Fig. 3. At *C* 1·5 g. of chloralose dissolved in 250 c.c. of water, were given through a stomach tube. At *P*, 55 min. later, 0·02 unit of "Infundin" was injected subcutaneously and caused maximal inhibition of water diuresis and albuminuria.

The intrathecal injection of "Infundin."

"Infundin," 0·1 unit, was injected intrathecally into a bitch which had been given chloralose in the manner described. The diuresis, which had well commenced, was completely inhibited for 2½ hours, at the end of which time the experiment was discontinued. Two days later, however, it was discovered that the intrathecal injection of 1 c.c. of physiological saline was equally effective in inhibiting water diuresis (Fig. 4). It is not easy to perform lumbar puncture in the dog, and it soon became evident that water diuresis was inhibited even though the needle did not enter the thecal canal. Subsequent experiments on the normal animal proved that attempts at lumbar puncture, if prolonged for from 5 to 10 min., invariably inhibited water diuresis in spite of the fact that the skin and subcutaneous tissues of the area pricked were previously infiltrated with a 2 p.c. solution of novocaine. Diuresis was usually completely inhibited for about 40 min. This result was obtained on many occasions and with five different bitches (Fig. 5).

On two occasions, the one animal being normal and the other under chloralose narcosis, the mere shaving of the back over the area of the

lumbar vertebræ was sufficient to inhibit water diuresis. It was thought that if the splanchnic nerves were blocked evidence might be obtained as to whether both the afferent and the efferent impulses were nervous. The attempt to block these nerves with 12 c.c. of a 1 p.c. solution of novocaine on each side was, however, in itself sufficient to inhibit water diuresis completely. It also became evident that this inhibition of water diuresis by afferent nerve stimuli was more readily caused in animals on which the

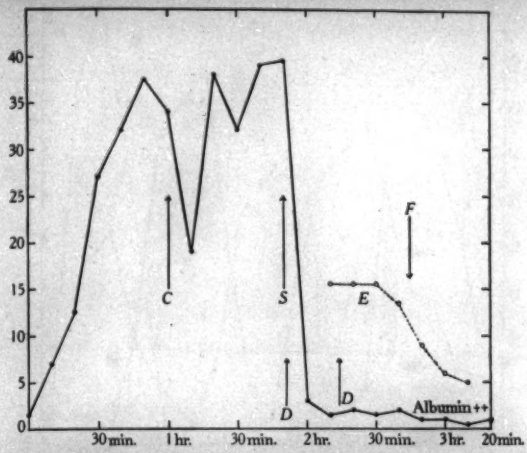

Fig. 4. Curve showing the effects caused by the intrathecal injection of 1 c.c. of physiological saline solution into a bitch under chloralose narcosis. Chloralose, dissolved in 250 c.c. of water, was administered through a stomach tube at *C*. The physiological saline solution was injected intrathecally at *S* and caused immediate and maximal inhibition of water diuresis. The animal defæcated at points *D*. Curve *E* represents the amounts of saliva collected, the secretion of which was inhibited by atropine injected subcutaneously at *F*. After diuresis had been inhibited the urine contained albumin.

"operation" had been performed more than once. In one animal it was found possible to cause a diminution of the rate of urinary secretion during diuresis by making ostentatious preparations for performing the puncture. These observations would suggest the possibility of setting up a conditioned reflex which would inhibit water diuresis.

It may therefore be stated that stimuli ranging in severity from shaving the skin to lumbar puncture inhibit water diuresis for varying periods of time, while the interval from the initiation of the afferent

nerve stimuli to the onset of the inhibition of the diuresis and the period of time for which the inhibition persists vary with the severity and the duration of the stimuli. Whether stimulation of the skin of the lumbar region is more likely to cause this effect than stimulation of any other area must for the present be left unanswered, but shaving the hair from the leg, which has been frequently performed, has on no occasion interfered in the slightest degree with water diuresis. The question as to

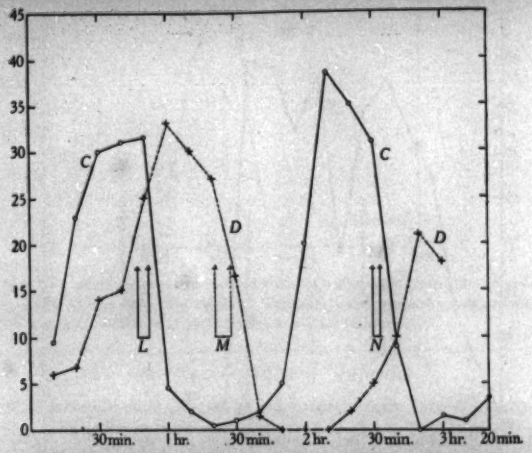

Fig. 5. Curves from two different animals showing the effects of afferent nerve stimuli on water diuresis. In curve *C* the arrows at *L* and *N* indicate respectively the points at which a needle was introduced into and withdrawn from the tissues between two lumbar vertebral spines. At *L* the duration of the stimuli was 5 min. and at *N* 3 min. The arrows at *M* have the same significance for curve *D*, the duration of the stimuli being 7 min. During the inhibition of diuresis which followed these stimuli, albumin was present in the urine secreted by both animals.

whether the inhibition of water diuresis caused in this manner is to be attributed entirely to nervous impulses will be considered in a subsequent paper. The only further point to be mentioned is that the urine, which at the height of diuresis resembled water, became thick and contained albumin after the stimuli had been applied.

It is evident that the phenomenon just described made it extremely difficult to disprove the assertion of Molitor and Pick that amounts of posterior pituitary extract which had no effect when introduced intra-

venously inhibited water diuresis when injected intrathecally. It was, however, possible on one occasion to show that ten times the amount of "Infundin" which definitely inhibited diuresis when given intravenously had no such effect when injected intrathecally (Fig. 6). On three other

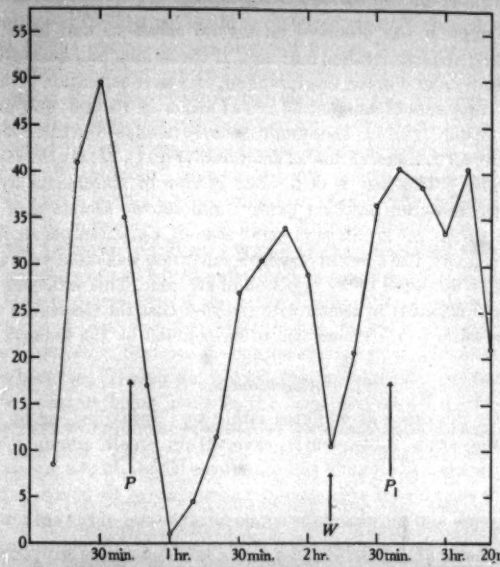

Fig. 6. Curves showing that the intrathecal injection of 0·05 unit of "Infundin" was without effect on water diuresis which was inhibited by the intravenous injection of one-tenth of that dose of "Infundin." At *P* 0·005 unit of "Infundin" was injected intravenously and caused minimal inhibition of water diuresis. The injection would appear to have been made when the rate of urinary secretion was falling, but the fact that the rate subsequently rose to 34 c.c. in 10 min. makes it clear that diuresis was effectually inhibited. At *W* a further dose of 250 c.c. of water was given. At P_1 0·05 unit of "Infundin" was injected intrathecally and was without effect on the rate of urinary secretion.

occasions it was found that a given amount of "Infundin" injected intrathecally inhibited water diuresis less effectually than when introduced intravenously (Fig. 2). Moreover, when 3 units of "Infundin" were injected intrathecally water diuresis was inhibited for 3 hours, whereas when the same amount was injected subcutaneously the inhibi-

tion lasted just over 4 hours. It may be concluded from these results that an amount of "Infundin" which when injected intrathecally has no effect on water diuresis may nevertheless be effective when injected intravenously.

Excessive salivation.

This symptom was observed on several occasions and in all three bitches while under chloralose narcosis. If the animal had been given an excess of water and diuresis was inhibited, excessive salivation invariably occurred. One animal secreted 35 c.c. of saliva in 15 min. and another 151 c.c. in 70 min. (Fig. 1). The symptom could be caused in three separate ways: (*a*) By an insufficient dose of chloralose (Fig. 1). (*b*) By the injection of "Infundin." This fact is of interest in view of statements by other authors that posterior pituitary extract inhibits the secretion of saliva [Gruber, 1929]. (*c*) By afferent nerve stimuli, *e.g.* attempts at lumbar puncture (Fig. 4). The onset of excessive salivation was often very sudden and could be inhibited by an injection of atropine. This vicarious excretion of water is hardly in accord with the view that the tissues are unable to liberate water to the circulation to be excreted by the kidneys.

Albuminuria.

If water diuresis was inhibited either by "Infundin" or by pathic stimuli, the urine subsequently excreted in small amounts almost invariably contained albumin and sometimes blood. It was interesting to observe the clear limpid urine excreted at the rate of 40–50 c.c. in 10 min. suddenly cease and the glass tube connecting the end of the catheter with the cylinder slowly becoming filled with opaque highly pigmented urine sometimes containing a considerable amount of albumin. So soon as diuresis recommenced the urine again became clear and contained no protein. These observations would suggest that the inhibition of water diuresis was effected by a direct action either on the kidneys or on the renal circulation.

DISCUSSION OF RESULTS.

It is clear that the experiments recorded do not support any of the conclusions reached by Molitor and Pick and indeed that the results obtained are directly contrary to theirs. The reasons for this divergence are:

(1) their experimental methods are open to serious objections;

(2) their conclusions are not altogether supported by the figures they published.

In the first place they made no effort to hydrate the tissues of the animals before obtaining their curves. In their early experiments food and fluid were withheld from the dog for 12 hours before the commencement of the experiment, while the practice observed in the experiments under discussion is not stated. The curves for water diuresis which they have published in their papers bear little resemblance to each other and still less to the normal curves obtained in this laboratory. It may be fairly claimed that they represent the measure of the dehydration of the tissues rather than that of water diuresis. Then, too, although they refer to the effects of posterior pituitary extract when injected intravenously, in practice all their injections were made subcutaneously. The rate of absorption of "Infundin" from the subcutaneous tissues is most variable and even such a large dose as 3 units may take 30 min. before it inhibits diuresis, if it be injected by this route. Further, Molitor and Pick injected the posterior pituitary extract at the same time that they administered the water. They therefore had no guarantee that water diuresis would have occurred even if the extract had not been injected.

A study of their own figures, moreover, makes it evident that they were not justified in drawing the conclusions they did. This study is made difficult because: (1) they did not adopt a standard dose of water, three separate amounts being given to the same dog on different occasions; (2) they used two different commercial preparations of the extract. It is stated that dog "Struppl," while under chloralose narcosis, excreted 54 c.c. of urine in 2 hours, after having been given 100 c.c. of water and 0·05 c.c. of "Physormon" subcutaneously, and that this amount was 72 p.c. of the normal (Protocol 2, p. 114). Elsewhere, however, it is stated that the same dog excreted 93 c.c. of urine in the same time after having been given the same amount of water (Protocol 6, col. 2, p. 119). Clearly 54 c.c. are not 72 p.c. of 93 c.c. Again it is recorded that "Jack" excreted considerably more urine than normal after being given 0·05 c.c. of "Physormon" subcutaneously (Protocol 5, p. 118), and yet just double this amount of the same extract injected by the same route reduced the amount of urine secreted in a given time to 25 p.c. of the normal (p. 115). That exactly half the amount of posterior pituitary extract that has such a marked and prolonged inhibitory effect on water diuresis should have no effect, other than to increase the output of urine, is contrary to our experience. Only one further example will be given. It is recorded that "Struppl" in normal circumstances excreted 106 c.c. of urine in three hours after having been given 100 c.c. of water (Protocol 6, p. 119). It is also shown that 41 c.c. of urine were excreted in the same time after

0·025 c.c. of "Physormon" had been injected intrathecally and the same amount of water had been administered. 41 c.c. thus represent 38·7 p.c. of the normal amount. At the foot of p. 114 it is stated that this dog only excreted 30 p.c. of the normal amount of urine after the same amount of "Physormon" had been injected subcutaneously. From their own figures, therefore, it is clear that the same amount of "Physormon" injected subcutaneously inhibited water diuresis as effectually as when injected intrathecally.

Summary.

1. Water diuresis which occurs while the dog is under chloralose narcosis is inhibited as effectually by the same amount of "Infundin" as when the animal is normal.

2. An amount of "Infundin" which when injected intrathecally has no effect on water diuresis may nevertheless be effective when injected intravenously.

3. An effective dose of "Infundin" when injected intrathecally does not inhibit diuresis for a longer time than when injected subcutaneously.

4. Water diuresis occurring in the dog, whether the animal be normal or under chloralose narcosis, may be inhibited by afferent nerve stimuli, varying in intensity from shaving the skin to lumbar puncture.

5. If water diuresis be inhibited, whether by the injection of "Infundin" or by afferent nerve stimuli, the urine subsequently passed in small amounts usually contains albumin.

6. The hypothesis of Molitor and Pick is not supported by these results, which are directly contrary to those they obtained and on which they based their hypothesis.

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ENERGY EXPENDITURE IN WALKING AND RUNNING.

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(*Received February* 28, 1934.)

It is well known that speed has a considerable influence on energy expenditure in walking and running. It is generally and correctly understood that, as a means of progression, walking is suitable for lower speeds up to a certain limit, while running is suitable for higher speeds. The average man cannot walk at very high speeds, nor can he execute the normal movements of running at very low speeds. The muscular mechanism of the body is adapted to run instead of walk when higher speeds are required, and to walk instead of run at low speeds.

There is, however, a range of speeds, within which walking and running are equally possible, and Benedict and Murschhauser [1915] and Furusawa, Hill, Long and Lupton [1924] have shown that, at such speeds running is more efficient than walking from the view point of energy cost. The former's results showed the energy expenditure of running to be about 15 p.c. less than for walking at a speed of 144 m. per min., while in the case of the latter, running at 163·8 m. per min. was only about 60 p.c. as expensive as walking at approximately the same speed.

In the present investigation the total energy expenditure and the oxygen requirement per minute were compared when walking and running at the same speeds. The oxygen requirement for the effort was measured as in the experiments of Furusawa, Hill, Long and Lupton, rather than the oxygen intake during the effort, which was the method of investigation adopted by Benedict and Murschhauser. The subject in the present experiments performed his exercise as naturally as possible, namely by walking or running a given distance on a horizontal track in the open air.

Experimental method.

The Douglas-Haldane method was used, the expired air of the subject being collected before, during and after each bout of exercise. The experiments were carried out in the morning, between 9.30 a.m. and 12.30 p.m. All experiments were made with the same subject, who is a laboratory assistant and a healthy, athletic young man of 26 years of age, 126 lb. in weight, 5 ft. 6 in. in height, and having a good deal of experience as a subject in metabolism experiments.

In order to ensure that the walking or the running should be as natural as possible, a course was set out on the roof of the School of Hygiene instead of using a treadmill in the laboratory. The course was 50 m. in length per lap and the subject walked or ran 500 m. or 10 laps in each experiment. It was decided not to have any artificial control of the speed which might absorb the attention of the subject when executing the effort. It is not easy to keep the same speed in walking and running in a natural way, but it was fortunately noticed in preliminary experiments that the subject showed nearly the same speed in walking as in running when he tried to walk as quickly as possible or to run as slowly as possible. This fact justified the subsequent carrying out of all experiments in such a way that the subject walked or ran at his quickest or slowest rate respectively, the actual speeds being accurately measured by stopwatch. The number of steps was counted during each 10 sec. of effort on five occasions per walk or run of 500 m., and the mean values were calculated in order to determine and check the rate of muscular movement per minute.

In addition to experiments with the quickest walk and slowest run, slower rates of walking and more rapid rates of running were also studied in a few experiments, for purposes of comparison and control.

Generally, two experiments were carried out in one morning, one walking and the other running, or in the reverse order on different days, so that any effects due to fatigue or training could be eliminated in averaging the results.

After a foreperiod of 30 min. resting sitting in a chair with feet up, the first collection of the subject's expired air was taken during 10 min. further resting in the same position. The subject was then fitted with a portable Douglas bag and took up his position at the start of the course to which he moved very slowly. At the moment he started to walk or to run the tap of the bag was turned on and the second collection of expired air commenced. After finishing the exercise the subject returned to the

chair and took up the resting position. The time of the second or exercise collection was in the majority of cases 5 min., *i.e.* 1 or 2 min. longer than the exercise itself. This collection was immediately followed by a third collection during a period of 30 min. throughout which the subject maintained the resting posture as before. On the conclusion of the recovery collection a further 10 min. collection was taken as a check on the resting value. Two sample tubes for each bag were analysed, the mean value for the percentage composition being used when the results differed in the two samples from a bag. As a general rule the difference did not exceed 0·06 p.c. in the two analyses, and experiments in which a greater difference appeared have been omitted from this paper. In some of the experiments with a very slow rate of walking the time of the exercise collection had to be slightly prolonged.

It will be noted that the base line from which the excess oxygen used for the exercise was assessed, was that of the oxygen consumption per minute of the subject when sitting resting in a chair with the legs raised. It would have been impossible to use the standing posture or work position for the resting and recovery collections owing to the length of time involved, for it has been noted that subjects vary a good deal in their ability to maintain a steady posture when standing and that fatigue materially influences metabolism.

Data of experiments.

The results are given in Tables I and II in which the experiments are listed in order of decreasing speed, except in the case of Exps. 15, 16, 17, 30 and 31, in which rubber shoes were worn.

The oxygen requirements in relation to speed is given in Fig. 1, from which it is clear that the difference between the oxygen requirement for walking and running increases with the speed of progression.

Discussion.

The data given in Tables I and II, and the curves in Fig. 1 show that when the rates of progression are substantially the same the increased oxygen requirement for walking as compared with running varies from approximately 4 p.c. at a speed of 121·1 m. per min. to 15 p.c. at 151·5 and 20 p.c. at 172·4 m. per min.

These results are similar to those of Furusawa, Hill, Long and Lupton, and are also in good accord with the findings of Benedict and Murschhauser. Benedict and Murschhauser determined the

TABLE I. Walking.

No.	Time, Speed and Step rate	Collection time min.	Ventilation l/min.	O_2 intake c.c./min.	R.Q.	O_2 requirement c.c./min.
1	2 min. 54 sec.	10 rest	4·40	228	0·81	4148
	172·4 m./min.	5	56·35	2140	1·12	
	198/min.	30 rest	6·99	309	0·94	
		10 rest	4·35	232	0·79	
2	2 min. 54 sec.	10 rest	3·99	229	0·81	4298
	172·4 m./min.	5	53·87	2110	1·13	
	198/min.	30 rest	6·60	331	0·96	
		10 rest	4·07	225	0·79	
3	2 min. 57 sec.	10 rest	4·47	233	0·81	4139
	169·5 m./min.	5	59·19	2127	1·31	
	194/min.	30 rest	7·07	322	0·99	
		10 rest	4·41	240	0·76	
4	3 min.	10 rest	4·00	221	0·81	4030
	166·7 m./min.	5	57·14	2081	1·15	
	190/min.	30 rest	6·79	314	0·94	
		10 rest	3·98	218	0·79	
5	3 min. 3 sec.	10 rest	5·41	247	0·87	3685
	163·3 m./min.	5	57·06	2077	1·15	
	186/min.	30 rest	8·50	315	0·91	
		10 rest	5·33	252	0·73	
6	3 min. 6 sec.	10 rest	5·87	238	0·83	3853
	161·3 m./min.	5	49·85	2029	1·07	
	190/min.	30 rest	7·76	313	0·89	
		10 rest	6·13	250	0·78	
7	3 min. 15 sec.	10 rest	4·20	237	0·80	3709
	153·8 m./min.	5	48·75	2098	1·10	
	186/min.	30 rest	7·97	325	0·91	
		10 rest	4·40	248	0·77	
8	3 min. 18 sec.	10 rest	4·31	227	0·81	3667
	151·5 m./min.	5	48·87	2110	1·06	
	186/min.	30 rest	8·65	318	0·93	
		10 rest	4·53	221	0·77	
9	3 min. 30 sec.	10 rest	4·65	241	0·81	3051
	142·8 m./min.	5	46·78	1941	1·10	
	180/min.	30 rest	8·24	310	0·90	
		10 rest	4·59	252	0·80	
10	3 min. 45 sec.	10 rest	4·51	240	0·79	2768
	133·3 m./min.	5	35·18	1968	0·92	
	174/min.	30 rest	6·78	298	0·89	
		10 rest	4·80	229	0·78	
11	4 min. 6 sec.	10 rest	4·10	231	0·82	2421
	122 m./min.	5	32·39	1942	0·90	
	144/min.	30 rest	5·77	272	0·93	
		10 rest	4·10	245	0·76	
12	4 min. 54 sec.	10 rest	4·08	229	0·82	1154
	102 m./min.	6	20·08	1050	0·84	
	132/min.	20 rest	5·96	258	0·92	
		10 rest	5·20	244	0·87	
		10 rest	4·14	227	0·79	

TABLE I (*cont.*).

No.	Time, Speed and Step rate	Collection time min.	Ventilation l/min.	O_2 intake c.c./min.	R.Q.	O_2 requirement c.c./min.
13	5 min. 15 sec.	10 rest	4·00	212	0·83	1216
	95·2 m./min.	6	21·60	1149	0·79	
	120/min.	20 rest	5·48	248	0·91	
		10 rest	4·47	216	0·80	
14	7 min. 33 sec.	10 rest	4·34	228	0·82	535
	66·2 m./min.	10	12·01	612	0·79	
	100/min.	20 rest	5·32	238	0·89	
		10 rest	4·31	227	0·80	
15*	3 min. 10 sec.	10 rest	4·94	225	0·83	3276
	157·7 m./min.	5	45·76	2058	1·10	
	185/min.	30 rest	6·73	264	0·92	
		10 rest	5·10	232	0·78	
16*	3 min. 12 sec.	10 rest	4·23	221	0·79	3181
	156·2 m./min.	5	49·18	1999	0·99	
	186/min.	30 rest	7·11	262	0·97	
		10 rest	4·58	227	0·75	
17*	3 min. 6 sec.	10 rest	4·93	235	0·82	3279
	161·3 m./min.	5	48·62	2040	1·07	
	190/min.	30 rest	6·99	273	0·92	
		10 rest	4·84	227	0·79	

TABLE II. Running.

18	2 min. 30 sec.	10 rest	5·10	240	0·85	3858
	200 m./min.	5	48·26	1921	1·11	
	168/min.	30 rest	6·33	279	0·85	
		10 rest	4·89	247	0·80	
19	2 min. 48 sec.	10 rest	4·48	228	0·89	3575
	178·5 m./min.	5	45·64	1996	1·09	
	156/min.	30 rest	5·97	266	0·92	
		10 rest	4·57	231	0·79	
20	2 min. 54 sec.	10 rest	4·10	221	0·82	3426
	172·4 m./min.	5	44·35	1928	1·04	
	162/min.	30 rest	6·50	266	0·98	
		10 rest	4·37	226	0·79	
21	2 min. 54 sec.	10 rest	4·57	230	0·81	3423
	172·4 m./min.	5	42·38	1981	1·00	
	160/min.	30 rest	5·99	269	0·97	
		10 rest	4·60	228	0·77	
22	3 min.	10 rest	4·38	230	0·81	3349
	166·7 m./min.	5	43·52	1957	1·10	
	160/min.	30 rest	5·99	277	0·96	
		10 rest	4·29	221	0·79	
23	3 min. 6 sec.	10 rest	4·72	238	0·88	3097
	161·3 m./min.	5	45·25	1978	1·10	
	160/min.	30 rest	6·10	268	0·96	
		10 rest	4·80	220	0·79	
24	3 min. 10 sec.	10 rest	4·02	229	0·85	3145
	157·7 m./min.	5	43·84	1997	1·01	
	156/min.	30 rest	5·12	266	0·99	
		10 rest	4·00	231	0·79	

* These experiments were carried out with the subject wearing a pair of light rubber shoes.

TABLE II (*cont.*).

No.	Time, Speed and Step rate	Collection time min.	Ventilation l/min.	O_2 intake c.c./min.	R.Q.	O_2 requirement c.c./min.
25	3 min. 15 sec.	10 rest	4·00	219	0·80	3112
	153·8 m./min.	5	42·90	1940	0·99	
	150/min.	30 rest	5·21	269	0·99	
		10 rest	4·11	220	0·78	
26	3 min. 15 sec.	10 rest	4·12	220	0·78	3108
	153·8 m./min.	5	41·66	1988	0·93	
	161/min.	30 rest	5·72	259	0·93	
		10 rest	4·40	229	0·79	
27	3 min. 18 sec.	10 rest	3·99	202	0·76	3119
	151·5 m./min.	5	40·15	1970	0·90	
	162/min.	30 rest	5·34	250	0·97	
		10 rest	3·89	203	0·82	
28	3 min. 30 sec.	10 rest	5·20	221	0·84	2646
	142·8 m./min.	5	38·05	1791	0·99	
	148/min.	30 rest	6·88	268	0·91	
		10 rest	5·01	219	0·81	
29	3 min. 40 sec.	10 rest	4·70	226	0·85	2738
	136·2 m./min.	5	39·04	1932	0·97	
	150/min.	30 rest	6·38	272	0·91	
		10 rest	5·12	240	0·81	
30*	3 min. 45 sec.	10 rest	4·10	222	0·84	2531
	133·3 m./min.	5	34·14	1868	0·89	
	150/min.	30 rest	6·30	263	0·95	
		10 rest	4·94	225	0·78	
31*	4 min. 7 sec.	10 rest	4·48	231	0·81	2306
	121·1 m./min.	5	35·84	1924	0·93	
	144/min.	20 rest	7·39	282	1·04	
		10 rest	4·93	235	0·82	

* These experiments were carried out with the subject wearing a pair of light rubber shoes.

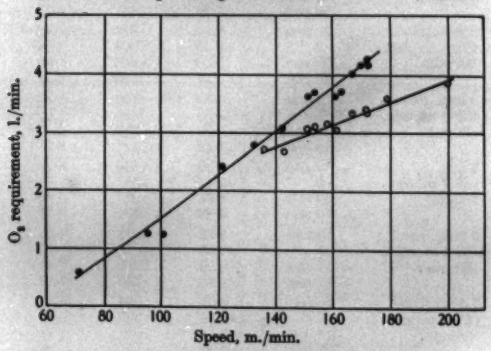

Fig. 1. Speed and O_2 requirement of walking and running.

•—• Walking. o—o Running.

oxygen consumption per minute as measured during the exercise; while in the experiments recorded in this paper the oxygen requirement was determined from the excess oxygen used in exercise and recovery. Benedict and Murschhauser's subject was no doubt in a steady state during the working collection, but at some of the higher speeds in the present study a steady state could not have been attained. The 5 min. collection of expired air, the exercise collection in the present investigation, included a minute or more recovery as well as the exercise, so that it is not possible to calculate the actual oxygen intake during exercise.

It must be borne in mind, however, that the above comparison of the oxygen requirement per minute for walking and running is not a true comparison of the oxygen used or required per unit muscular movement, namely per stride, when walking and running. In the case of running fewer movements are performed than in walking although the speed of progression and the total distance covered may be the same. Calculations of the actual excess oxygen required per stride show that at the same speed of progression, whether in walking or running, each stride necessitates approximately the same expenditure of energy as judged by the oxygen requirements. Inasmuch as the length of stride in walking is less than in running more strides must be taken to maintain the speed and cover the distance, and the difference in oxygen requirement appears to be directly related to the number of strides taken in each case. Table III shows a comparison of the oxygen requirements per stride when walking and running at various speeds.

Table III. Oxygen requirement (c.c.) per stride in walking and running at various speeds.

Speed m./min.	Walking	Running c.c.	Speed m./min.	Walking	Running c.c.
200·0	—	23	151·5	20	19
178·5	—	23	142·8	17	18
172·4	21·5	21	136·2	—	18
169·5	21	—	133·3	16	17*
166·7	21	21	122·0	17	—
163·3	20	—	121·1	—	16*
161·3	19	19	102·0	9	—
157·7	18*	20	95·2	10	—
156·3	17*	—	66·2	5	—
153·8	20	20			

* These figures were obtained when the subject wore a pair of light rubber shoes.

A strict comparison of walking and running could only be made if the same length of stride and frequency of stride were adopted in each case, but as this is almost impossible and certainly unnatural at high

speeds the results of such an experiment would be of little if any significance.

The curves shown in Fig. 1 for the oxygen requirement in relation to speed and Fig. 2 for the number of strides taken in walking and running in relation to speed, possess similar characteristics, and it is

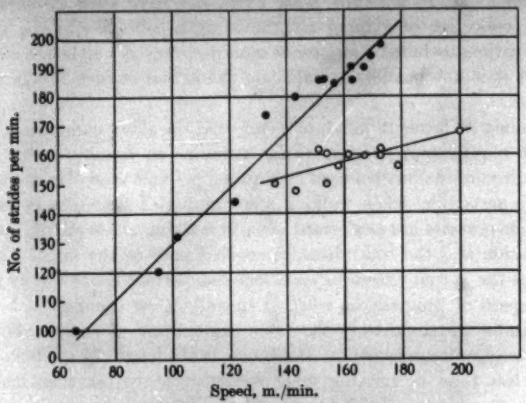

Fig. 2. Speed and stride frequency for walking and running.
•—• Walking. o—o Running.

therefore thought that the increased oxygen required per minute for walking at high speeds as compared with running is substantially due to the fact that more muscular movements are exerted per minute in order to maintain the same rate of progression.

Influence of footwear.

In some experiments which I have carried out marked differences have been found in energy expenditure when light rubber or heavy walking shoes were worn. It may be pointed out in this connection that Exps. 15, 16 and 17 (Table I) and 30 and 31 (Table II) were carried out with the subject wearing a pair of light rubber shoes instead of the leather walking shoes which were used in all the other experiments. In these experiments using rubber shoes the oxygen requirement for the effort was less than that when leather shoes were worn, particularly so in the case of the walking experiments. Difficulty was found by the subject in running at low speeds unless rubber shoes were worn.

Influence of arm swing.

Benedict and Murschhauser claimed that the arm swing in walking caused a marked increase in energy expenditure, and attempted to allow for the effect of arm swing in their computations of the energy expenditure by determining the oxygen used by their subject when standing and swinging the arms as in walking. They stated that with the oxygen consumption for arm swing as the base line the energy cost of walking proved to be some 3 p.c. less than for running at the same speed.

In walking at high speeds the arms must be swung quite vigorously across the chest in order to provide a reaction so that the opposite leg can be brought forward without bending at the knee as in running. Otherwise, at high speeds, walking degenerates into running. The extra expenditure in walking may possibly be due partly to the swinging of the arms, and also partly to the maintained contraction of the muscles of the leg, but the actual number of muscular movements appears to be the main cause.

I carried out a few experiments to determine the energy cost of arm swinging at the rates executed when walking, and the results showed the oxygen requirement per minute for arm swing to be on the aggregate 906 c.c. for 3 min. 6 sec., swinging at the rate of 190 arm swings per min. Subtracting this figure for the oxygen requirement per minute of standing arm-swinging exercise, the energy cost of the remaining movements of progression in walking at the speed of 161·3 m. per min. would be about 11 p.c. less than the cost of running at the same speed—a result which appears difficult to believe.

As the accurate estimation of the oxygen requirement for arm swinging alone is practically impossible, owing to other factors increasing oxygen usage, as for example postural balance and increased respiratory movements, I do not think that this result is of any value. Moreover, inasmuch as arm swinging is an essential feature of walking at high speeds the isolated study of the movement is without significance in comparing the relative oxygen requirements for walking and running at the same speed.

Summary.

The oxygen requirements for walking and running at the same speed under natural conditions in the open air have been investigated by the Douglas-Haldane method. The results are similar to those of

Furusawa, Hill, Long and Lupton, and in good accordance with the laboratory treadmill experiments of Benedict and Murschhauser.

With increasing speed of progression the oxygen requirement for walking increases at a greater rate than for running. The increased oxygen requirement for walking as compared with that for running at the same rate varied from 4 p.c. at a speed of 121·1 m. per min. to 15 p.c. at 151·5 and 20 p.c. at 172·4 m. per min.

It was noticed in walking at high speeds that the oxygen requirement for the effort was less when light rubber shoes were worn than when leather walking shoes were used.

The vigorous arm swinging across the chest adopted when walking at high speeds may necessitate increased oxygen usage, but it is not thought to be a material cause of the difference in oxygen requirement observed in walking and running at the same rates of progression. The isolated study of the energy expenditure in arm swinging is considered to be of little if any significance.

The oxygen requirement per stride when walking or running at the same speed is shown to be substantially the same, and therefore it is thought that the increased oxygen requirement for walking is essentially due to the fact that more muscular movements are necessary because the length of stride is less.

I should like to express my deep indebtedness and thanks to Dr G. P. Crowden for his kind help, without which this investigation could not have been carried out.

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612.143

A DEVICE FOR ESTIMATING BLOOD-PRESSURE IN THE RABBIT[1].

By R. T. GRANT and P. ROTHSCHILD.

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(*Received March* 1, 1934.)

The following simple device has been found serviceable for estimating the systolic blood-pressure in the central artery of the ear in the normal and unanæsthetized rabbit. It is based on von Recklinghausen's method [1906] and seems to us more satisfactory than any other we know; references to similar devices will be found in the papers by Kottlors and Rothschild [1932] and by Rai [1930–1]. When proper precautions are observed, the systolic pressure measured in the ear artery can be taken as a reliable index of systemic arterial pressure.

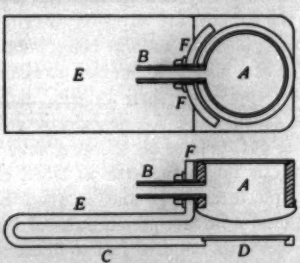

Fig. 1.

The device consists of an airtight pressure capsule and a support by which it is applied to the ear; it is shown in plan and in vertical section in Fig. 1 (about nat. size). The capsule *A* is a cylinder of aluminium tubing 10 mm. long and 19 mm. outside diameter, the thickness of the wall

[1] Work undertaken on behalf of the Medical Research Council.

being 1·5 mm. The upper end of the cylinder is recessed internally, and into this is cemented a glass circle; the lower end is grooved externally and is covered by Cargile membrane which is tied on to this groove. The membrane is lax. A brass tube *B*, 3 mm. outside diameter and bore 1·5 mm., length 15 mm., is screwed into the middle of one side of the capsule; on the outside of the tube a screw thread is cut. The support is a U-shaped strip of aluminium 1·5 mm. thick and 20 mm. wide; the gap between the limbs of the U is 2 mm. The lower limb *C* is 55 mm. long and into its free end (beneath the capsule) is inserted a glass circle *D* 16 mm. diameter. The upper surface of the glass is flush with the aluminium. The upper limb of the support, *E* and *F*, is 40 mm. long; 10 mm. from its free end two cuts, each 5 mm. long, are made at each side. The last 10 mm. of aluminium, *F*, are turned vertically upwards at these cuts and then bent into an arc of a circle to fit the outer side of the capsule. The capsule is held firmly to the aluminium arc by a nut and washer on the brass tube. The capsule tube is connected by rubber tubing to a bottle of 2 litres capacity and through this to a mercury manometer and rubber pressure bulb.

To use the capsule, either the ear is previously epilated or the hair is cut short over the central artery towards the base of the ear. The membrane of the capsule is moistened with 70 p.c. glycerine. The thin postero-lateral margin of the ear is slipped between the limbs of the support until the central artery lies beneath the centre of the capsule. When the limbs of the support are adjusted to grip the ear gently, the light capsule remains in position without being held by hand unless the animal is restless. The ears may be kept erect by fastening them together with adhesive plaster applied to their anterior margins. The artery is viewed through the capsule and the pressure is gradually raised. The lax membrane, filling out, presses on the skin over the artery. As the intracapsular pressure is raised the arterial blood column becomes reduced in diameter, pulsates freely and is then interrupted first in diastole and finally, after a further pressure rise of 1 or 2 mm., in systole also. The pneumatic pressure required just to break the column of blood in systole is taken as the systolic pressure in the artery. This end-point is readily determined and, if respiration is not too rapid, even the small rise and fall of blood-pressure with expiration and inspiration can be observed.

It is to be remembered that the ear artery is small and, under ordinary conditions of body activity and temperature, is subject to great fluctuations in calibre; thus the column of blood measuring, for example,

1·0 mm. in diameter when the artery is dilated, may be reduced to only 0·1 mm. when the artery is greatly constricted by cooling or startling the animal. Comparison of the ear pressure in the anæsthetized animal with that measured by mercury manometer through a cannula in the carotid or femoral artery (two observations each) shows that, provided the ear artery is not greatly constricted, systolic ear pressure lies 10 to 15 mm. below mean carotid pressure, and is the same as mean femoral pressure. As has been noted by previous observers, the required degree of arterial relaxation is attained by keeping the animal sufficiently warm. It may also be attained by denervating the ear as has been done by Kawaguchi [1931]; the ear, however, must be freshly denervated for, if the nerves have been cut more than 4 or 5 days previously, the vessels may be greatly constricted, unless the animal has been resting quietly for some time [Grant and Bland, 1931–3]. In the unanæsthetized animal, warm and resting quietly, the blood-pressure remains almost steady for long periods, and usually lies between 70 and 90 mm. Hg; in an excited or restless animal the pressure may rise to 100 mm. Hg or over. When care is taken to make estimations under standard conditions of rest and warmth the pressure remains remarkably constant from day to day. Considerable constriction of the ear artery materially lowers the pressure necessary for occlusion. Thus, in a rabbit with one ear normal and the other freshly denervated, the pressures were measured simultaneously with a capsule on each ear. The pressures in the two ears were the same when the animal was warm, the vessels of both ears being equally relaxed. When the animal was cooled the vessels of the normal ear constricted greatly and the pressure in this ear fell ultimately to 10 mm. below its previous level; the vessels of the denervated ear remained unchanged and the pressure here rose 10 mm., presumably the result of vaso-constriction elsewhere. There was thus a pressure difference of 20 mm. Hg between the two ears. On re-warming, the vessels of the normal ear relaxed and the pressures in both ears returned to their previous level. The fall of pressure in the constricted artery is interpreted as being due to increased resistance to the blood flow by the narrowing of the vessel lumen. For, when the artery is dilated, there is little difference (up to 10 mm.) in the pressures measured at the base and tip of the ear, but when the artery is greatly constricted the tip pressure may be as much as 35 mm. Hg below that at the base, this fall of pressure taking place over a 5 cm. length of artery. It may be noted that Wishart [1933] has recently recorded similar alterations in the gradient of systolic pressure in man between the wrist and finger vessels;

with vaso-dilatation the wrist-finger systolic difference diminished from 40 to 14 mm. The human digital arteries are comparable in size with the ear artery of the rabbit.

The state of the smaller vessels of the ear has a definite though slight effect on the systolic pressure in the main artery; constriction causing a rise and relaxation a fall. For example, in the cooling experiment just referred to, the fall of pressure in the normal ear was preceded by a rise; this occurred as the ground tone of the ear blanched, owing to constriction of the minute vessels, and before the main artery became obviously reduced in calibre. So also a subcutaneous dose of adrenaline, acting slowly on the vessels, gives first a rise of pressure when the minute vessels constrict, followed by a fall when the dose is sufficient to constrict the main artery. Again, we have noted that in a warm and resting animal with one ear freshly denervated and the vessels of the normal ear dilated, the pressures in the two central arteries are equal; after a few days, when tone has been regained in the denervated ear and its ground colour is paler than that of the normal ear, the pressure in the artery of the denervated ear is usually a little higher (5 mm. Hg) than that in the normal ear. Similarly, occlusion of the central artery of the normal ear distal to the capsule raises the systolic pressure slightly. Again, the slight fall of pressure that is found when estimations are made in rapid succession is to be attributed to relaxation of the distal vessels. When the animal is warm or an ear is freshly denervated, the main ear arteries are fully relaxed but the minute vessels though dilated are not maximally so. They can be opened further in several ways, for example, by occluding the central artery for even a few seconds; when the artery is released the ear becomes temporarily more flushed (reactive hyperæmia). This flushing of the ear is seen when the pressure is released in the capsule after the artery has been occluded and, if further compressions are made before the flush subsides, the obliteration pressure falls to reach a level usually about 5 mm. below that estimated at the first compression. If a sufficient interval (about 1 min.) is allowed between the readings for the vascular reaction to subside, no pressure fall occurs.

Another use of the capsule is to count the heart rate; this is more easily done by viewing the pulsations of the partially compressed artery than by palpation of the carotid or femoral vessels, specially when the rate is rapid.

The device described is one which, from several points of view, is of value in studying changes in the circulation of its normal unanæsthetized animal.

SUMMARY.

1. A simple device is described for estimating systolic blood-pressure in the ear of the rabbit, normal and unanæsthetized.

2. With proper precautions, systolic pressure in the ear is a reliable index of systemic arterial pressure.

3. Great constriction of the artery itself lowers systolic pressure considerably; dilatation of more distal vessels lowers, while constriction raises, systolic pressure slightly in the ear artery.

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612.141

THE RESISTANCE OF ARTERIAL WALLS AND ITS EFFECT ON BLOOD-PRESSURE READINGS.

By I. HARRIS.

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(*Received March* 1, 1934.)

For some years I have attempted to obtain arterial pressure readings by employing a method which gradually raises the pressure in the veins in the lower arm to that of the level of the brachial artery. I made use of this method mainly for estimating the pressure required to overcome the resistance of the arterial wall. My attention has recently been drawn to the fact that methods based on similar principles have already been published. It appears that Hill and Flack [1909] were the first to employ this principle for the purpose of blood-pressure determinations.

"We place one armlet round the brachial, and another narrower one round the forearm of the same arm. We find the obliteration pressure with the first armlet. Suppose it is 150 mm. Hg. We lower the pressure in this armlet to, say, 145 mm. Hg, so that the arterial blood can get through into the limb, but cannot get out of the veins of the limb until the pressure in the veins rises above 145 mm. Hg. Allowing sufficient time for the veins to fill, we then measure the pressure in one of the superficial veins, and find that it does finally reach this pressure thus: We raise the pressure in the second armlet above 145 mm. Hg, and choosing a suitable vein above this armlet, by stroking empty it upwards past the next valve, then quickly lowering the pressure in the second armlet observe the pressure at the moment when the vein fills from below. Now if we find the pressure in the vein reaches 145, we know that the obliteration pressure was correct within 5 mm. Hg...."

De Vries Reilingh [1913] adopted a different procedure for the purpose of obtaining pressure readings by means of a method based on the same principle. He employs the Riva Rocci apparatus to compress the artery and, at the same time, he uses a plethysmograph for the purpose of determining variations in the pressure below the armlet. This

author found that the pressure readings obtained by means of this plethysmograph differ frequently from those obtained by the usual method of the Riva Rocci apparatus. The latter method gives mostly higher figures than the former, which he accounted for by the fact that the external obliteration pressure measures the arterial pressure plus resistance of arterial wall. In some instances, however, pressure in the veins was higher than the obliteration pressure in the armlet of the Riva Rocci apparatus. His explanation of this paradoxical phenomenon is as follows: The arterial pressure was at one time higher during the early part of the experiments, and the valves of the veins prevent back flow of blood from these vessels.

That the method employed by this author is not quite reliable follows from the fact that he estimated the average resistance of the walls of normal vessels at 19 mm. of Hg, which is doubtless far too high.

Zarday and Razgha [1931], to whom the work of the two previous authors was apparently unknown, employed a procedure based on the same principle. They used the Riva Rocci apparatus for compression of the arteries, and in the first instance they used the Gartner tonometer to determine variations of pressure in the veins. Later on they inserted a cannula into a vein, but I am not aware that they obtained graphic records of their observation. The findings of these authors on the whole agree with those of Reilingh, but their interpretation of these phenomena differs completely from his. According to them the usual determination of the Riva Rocci method does not measure at all the force required to obliterate the artery, but simply determines the force required to transform rhythmic pulsations into a constant flow. In other words, after obtaining the maximum systolic pressure by means of the Riva Rocci apparatus, blood still trickles through the obstruction into the vein until the pressure in the vessels reaches a certain constant level. The true arterial pressure, which is really the aortic pressure, can only be obtained by measuring the pressure in the veins under the arrangement as just stated. These authors speak of a column of blood which stretches itself from the aorta to the obliterating point of the vein and the pressure of which remains constant from one point to another. It is clear, if these authors are right in their interpretation of these phenomena, that the principle of this method cannot be used to estimate resistance of the arterial wall, since the determinations by the Riva Rocci armlet do not give systolic pressure readings at all.

That the interpretation of these authors is untenable follows from the following:

(1) The exact time of closure of the artery by means of the armlet of

the Riva Rocci apparatus is readily ascertainable. As soon as the artery is completely obliterated there follows a fall of pressure in the veins. (See Fig. 1.) Zarday and Razgha have also noticed the sudden lowering of the pressure of the vein as a result of arterial obliteration. There is no doubt at all that this depression of the venous curve as recorded in the graph coincides with complete obliteration of the artery by means of the armlet. It usually takes a considerable time before the blood accumulates in the veins in sufficient quantity to equal the arterial pressure. If the artery is obliterated early, this fall in the venous pressure manifests itself all the same. There is no rise in the venous pressure as long as the pressure in the armlet is not lowered. As soon, however, as the pressure in the armlet sinks, the pressure in the veins rises. This can be repeated *ad lib.* with invariably the same result. (The fall of the pressure in the veins after the closing of the arteries may be due to exudation of fluid into the tissues, etc.) On the graphs the obliteration pressure can be read with exactness within the margin of error of a few mm. of Hg. Table I gives the obliteration pressure of the brachial artery taken from the graphs, and compared with the pressure readings taken in the usual way by the Riva Rocci instrument (auditory method) before the patient was examined by the method here described. It will be seen that, considering the various factors which influence pressure readings, the two sets of figures run closely together. We may safely conclude that the determination of systolic pressure by the Riva Rocci instrument means complete obliteration of the brachial artery.

Fig. 1. Upper curve = blood-pressure curve by the ordinary method. Lower curve = intra-arterial pressure. 1 = basal line for lower curve. 2 = basal line for upper curve.

(2) It will be seen from Table III that amyl nitrite reduces the difference between the external pressure as applied and the pressure obtained directly from the veins. This suggests that the tonus of the vessels accounts, to some extent at any rate, for the different readings of pressures obtained by the two methods.

(3) There remain to be explained instances in which the compression pressure is lower than the one in the veins below the armlet. I will deal with this in a separate paragraph at a later stage.

It may therefore be of some interest to give a series of determinations of the degree of resistance in the arterial wall by a procedure which gives more exact readings than it was possible to obtain by the older methods.

TABLE I.

Case	R.R.	V.P.	Case	R.R.	V.P.
1.	120	124	11.	160	162
2.	140	150	12.	200	192
3.	185	186	13.	170	170
4.	150	142	14.	120	124
5.	240	256	15.	140	150
6.	140	139	16.	185	186
7.	130	126	17.	120	126
8.	175	164	18.	155	150
9.	105	109	19.	120	126
10.	115	115			

R.R. = Riva Rocci.
V.P. = Highest pressure in the veins just before occlusion of the arteries.

TABLE II.

Case	O.P.	V.P.	D.	
1.	147	133	14	Combined valvular disease
2.	293	281	12	High blood-pressure
3.	139	131	8	Normal
4.	248	232	16	High blood-pressure
5.	267	252	15	High blood-pressure
6.	126	114	12	Hyperthyroid
7.	153	150	3	Functional cardiac condition
8.	164	148	16	High blood-pressure
9.	116	126	−10	Degeneration of myocardium
10.	165	178	13	Aortic regurgitation
11.	108	97	11	Hypotony
12.	119	117	2	Cardiac neurosis
13.	189	179	10	High blood-pressure
14.	146	133	13	Cardiac insufficiency
15.	127	112	15	Combined valvular disease
16.	164	141	23	High blood-pressure
17.	211	187	24	High blood-pressure
18.	134	125	9	Nephritis
19.	132	122	10	Combined valvular disease

O.P. = Occlusion pressure by means of the Riva Rocci armlet.
V.P. = Highest pressure in the veins just before occlusion of the arteries.
D. = Difference between the two pressures.

TABLE III.

	Before amyl nitrite			During amyl nitrite			Soon after amyl nitrite		
Case	Armlet pressure	V.P.	Difference	Armlet pressure	V.P.	Difference	Armlet pressure	V.P.	Difference
1.	147	133	14	122	116	6	152	136	16
2.	142	124	18	119	108	11	126	113	13
3.	147	122	25	90	89	1	103	90	13

Method.

The principle of this method has already been indicated. If an armlet is placed on the upper arm and the pressure is gradually increased till the artery is obliterated, the venous pressure in the distal end of the arm will gradually rise until the moment before compression of the artery is complete it will equal the intra-arterial pressure. The following arrangement was made use of: Two mercury manometers were adapted to register in the usual way on a rotating drum covered with smoked paper. One of these manometers was connected with the armlet of the Riva Rocci apparatus placed on the upper arm. The other one was connected by means of a needle to the vein. Between the venous pressure recorder and the mercury manometer was an uninterrupted column of fluid consisting of physiological saline and an anti-coagulant. Both pressures were recorded simultaneously on the same recording surface. (See Fig. 1.) The pressure in the armlet was raised in some instances by 10 mm. of Hg at a time, in others by 5 mm. As soon as the artery is completely obliterated there is invariably a fall in the venous pressure. This feature was taken as an indication that the artery is compressed. The level of the upper curve of the graph just before the rise of pressure which obliterated the artery was taken as index of the systolic pressure. It will therefore be seen that there is a margin of error of a few millimetres of mercury in the readings. Whilst compressing the arm care has been taken that the artery is not completely obliterated before sufficient time has elapsed to allow the blood of the artery to reach the vein. This time is variable and it is not easy to tell whether the pressure in the veins has fully reached the highest possible level before the artery is completely occluded. It is not therefore safe always to rely upon one reading. After the first reading has been taken the pressure in the armlet should be somewhat lowered below the obliteration point and, after an interval, a reading taken again. Only when the venous curve remains somewhat on the same level may we be sure that there is a complete equilibrium between the pressure in the veins and that of the intra-arterial pressure.

Coagulation of blood in the veins is another pitfall which however is not difficult to avoid.

Results.

Table II shows the pressure required to overcome the resistance of the arterial walls on various levels of systolic pressures. It will be seen that the resistance of the arterial walls has very little influence on total pressure reading. It may be said that there is a tendency for the re-

sistance of the walls to become greater in cases of hypertony, but in the main it must be admitted that the resistance of the arterial walls does not account to any great extent for the pressure readings in abnormally high cases of hypertony. The clinical facts must be taken into consideration, that there are instances of low pressure with arterial walls that are obviously thickened. Apparently in normal subjects the resistance of the walls is very slight.

The problem still remains, how far the resistance of the arterial walls is due to arterio-sclerosis or tonus. Table III demonstrates without doubt that the tonus accounts in some degree for the resistance of arterial walls. It is, however, impossible to say to what extent a given dose of amyl nitrite will do away with the tonus. Five minims of amyl nitrite were inhaled in this experiment.

The real difficulty is to find an interpretation for the fact that there are instances in which the pressure in the veins is higher than the obliterating pressure. From a clinical point of view those cases were suffering from advanced cardiac disease. I will give here the readings of a typical case.

	Pressure in the armlet	Pressure in the veins
	107	117
	116	126 (artery obliterated)
After lowering pressure	114	126
	116	127
	125	135 (artery again obliterated)

De Vries Reilingh's explanation that in this instance the arterial pressure might have been higher at an earlier stage of the observations cannot possibly account for the higher pressure in the veins in this instance, because the arterial pressure through the whole experiment was never raised to the level of the venous pressure. To my mind the whole difficulty resolves itself into a very simple problem. Here is a case where the pressure in the veins below the armlet is about 10 mm. higher than the pressure which prevents the flow of blood from the lower arm through the obstruction caused by the armlet. We all know that such a condition is impossible. As soon as the pressure in the veins below the obstruction reaches a slightly higher level than the pressure in the armlet, the obstruction would be removed until the level of pressure below the armlet equals the obliterating pressure. It ought to be physically impossible, apart from other considerations, to have a pressure in the veins below the armlet 10 mm. higher than the pressure in the armlet. I suggest that in these cases, owing to back pressure in the veins, brought about by heart failure, there is considerable pressure in the veins above the armlet which

must be added to the pressure in the armlet. Therefore, in order to overcome the obstruction of the armlet from below, it is necessary to employ a pressure equal to the pressure in the armlet plus the pressure in the veins above the armlet.

This only deals with one side of the problem. The question still remains—How is it possible for the pressure in the vein to be higher than that of the arterial pressure? The fact must be kept in mind that all the cases which show these phenomena are advanced cases of heart disease with heart failure. From the very first when the armlet was applied the pressure in the veins was greater than that in the armlet. It must also be kept in mind that the heart acts as a pump, and that veins have valves which prevent regurgitation towards the capillaries. In normal cases the veins fill but slowly after compression, and if for some reason there is momentarily a higher pressure in the veins than in the armlet, the venous circulation towards the heart will be restored till the pressure in the veins below the armlet about equals that of the armlet. In such conditions, just before the arteries are completely obliterated the action of the pump ceases to be effective, and there will simply be a flow of blood from a higher to a lower level of pressure till an equilibrium is attained. In such circumstances the lower level of pressure is usually that of the veins which, as we have seen, fill but slowly. In cases of heart failure the conditions are different. The venous pressure is abnormally high before the armlet is applied. Additional fluid is pressed out owing to the action of the heart. Just before the arteries are compressed the effects of the cardiac force cease. The pressure in the veins under such conditions would be higher than that of the arteries, but no equilibrium between the pressures is possible owing to the existence of valves in the vein which prevent regurgitation. But whatever the explanation of this phenomenon is, it occurs only in cases of heart failure, and therefore this problem does not arise in normal cases.

Conclusion.

The resistance of the arterial walls has little influence on total pressure readings.

In part, at any rate, the resistance of the arterial wall is due to tonus.

I am greatly indebted to Prof. Roaf for the interest he took in the problem, for the loan of apparatus and for very valuable suggestions.

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612.627

RECIPROCAL ACTIVITY OF THE CORNUA AND CERVIX UTERI OF THE GOAT.

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(*Received March* 5, 1934.)

In this paper are recorded a few experiments on smooth muscle taken from the pregnant uterus of the goat; they suggest a reciprocal activity which is nervous in origin on the part of the fundus and cervix uteri. It is not proposed to mention the history of this subject, except to say that no record has been found of any similar experiments having been made previously.

Method.

Strips of uterine muscle were suspended in a bath of oxygenated Dale-Ringer solution at 37° C., and their contractions recorded on a slow drum by means of a light lever and frontal writing point. The pregnant goats supplying the strips were the subjects of experiments by Prof. J. Barcroft [1933], involving removal of fœtuses through a uterine incision; the whole of these operations were performed under saline solution at 37° C., the goat, anæsthetized with urethane (4 c.c. of saturated solution per kg.) and chloroform-ether mixture as required, lying supine in a large bath. At the close of the experiment longitudinal strips were cut from each of the cornua (previously intact) and the whole cervix excised. These three pieces of tissue were placed in a specimen tube with cotton wool soaked in saline, the tube packed in ice and despatched from Cambridge to London. The strips from the cornua were trimmed, washed, and suspended in separate baths. The cervix was sliced transversely, the resulting rings opened, cleaned as well as possible, and suspended in further baths. This was about 3–3½ hours after their excision from the living uterus.

Results.

Specimens were obtained from six goats, respectively 13½, 14½, 15½, 16½, 18 and 19½ weeks' pregnant (full term is about 21 weeks). Their serial numbers [Barcroft, 1933] were 9, 19, 11, 12, 14 and 15 respectively.

Cornua. The strips went into sustained tonic contraction in the bath, small spontaneous contractions being superimposed; these were larger and less frequent in the last goat, nearing full term, but the conditions of the experiments do not allow of definite significance being attached to this. 0·02 unit of *oxytocin* had a just perceptible effect in all cases, either in increasing the tonus, augmenting the contractions, or both. *Adrenaline* (final concentration 1 in 1 million) caused general relaxation, and slowing or stoppage of the spontaneous contractions. *Acetylcholine* (final concentration about 1 in 100 million) produced contraction and augmentation of the rhythm.

The goat has button placentæ, and although the majority of these are found in the body of the uterus, some are usually situated in one or other of the horns. No significant or consistent difference was noticed in any of the above particulars between the horn containing placentæ and that from which they were absent.

Cervix. This in all cases was plugged with a quantity, greater in the later animals, of extremely sticky mucus. It was almost impossible to free the circular strips from this without damaging them, so that a certain amount must always have been present in the bath. In the successive animals the cervix grew shorter and more spongy, so that in the protocol of the first experiment its consistency is described as "cartilaginous," and in that of the last experiment as "fairly soft."

In four experiments minute spontaneous contractions were observed; their absence in the other two probably has no significance.

Oxytocin had no effect on these strips, even in amounts up to 2 units. Owing to the unpromising nature of the strips and the uninteresting tracings which they provided, this would not have been remarkable except in view of the following findings. *Adrenaline* caused a contraction of the strips, as did *acetylcholine*. In one experiment the acetylcholine contraction was superimposed on that due to adrenaline. As can be seen from the figures, the effective power of contraction of the cervical strips is small, but these changes in length were quite obvious, and in view of the inactivity of oxytocin, striking.

With the possibility in mind that this might be an example of a general reaction of the circular muscle, one strip was cut from a horn in a

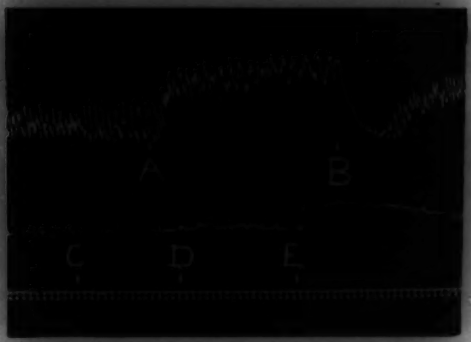

Fig. 1. Goat 11. 15 weeks' pregnant. *Upper tracing:* longitudinal strip of uterine horn in 50 c.c. bath. At A, 0·02 unit oxytocin; at B, 0·03 c.c. 0·1 p.c. adrenaline. *Lower tracing:* circular strip of cervix uteri in similar bath. At C, 0·02 unit oxytocin; at D, 2·0 units oxytocin; at E, 0·03 c.c. 0·1 p.c. adrenaline. Time tracing in minutes.

Fig. 2. Goat 14. 18 weeks' pregnant. *Upper tracing:* longitudinal strip of uterine horn in 50 c.c. bath. *Lower tracing:* circular strip of cervix in similar bath. At A, to both strips, 0·04 c.c. 0·1 p.c. adrenaline. At B, to both strips, (about) 0·5γ acetylcholine in 0·1 c.c. saline. Time tracing in minutes.

circular direction. Adrenaline did not cause relaxation of this strip, but abolished all rhythmic contractions. This provides no evidence for associating the circular muscle of the cervix with that of the remainder of the uterus.

These experiments were arranged so that at one time or another adrenaline, acetylcholine and oxytocin were each allowed to act on previously untreated strips. Whether or not the strips were untreated, the action of these substances remained constant.

Discussion.

Owing to the interval elapsing between the excision of the muscle and its mounting in the bath, it is not permissible to draw any conclusions from differences in spontaneous activity observed in the strips from the various goats. In view of the findings of Robson [1933*a*, *b*] and others in more careful experiments than these, it is proposed to ignore also the quantitative aspect of the reaction of the horns to oxytocin with advancing pregnancy. Since no parasympathetic nervous supply to the uterus has been described, the experiments with acetylcholine merely demonstrate at present that contraction can be produced in the cervical strips by other means than the administration of adrenaline.

We are left, therefore, with two constant findings which are well marked, and unlikely to have been influenced qualitatively by the experimental conditions. The first of these is that whereas the cornua contract with a small dose of oxytocin, the cervix appears insensitive even to amounts which are almost ridiculously large. The second is that adrenaline causes a relaxation of the horns and contraction of the cervix.

It is surprising that the cervix should be insensitive to oxytocin; the reaction of the strips to adrenaline and acetylcholine shows that the smooth muscle in the preparation was sufficient to produce a registrable contraction. The low threshold of the cornual muscle discounts any general hormonal depressant, and we are faced with two alternatives. Either the circular cervical muscle is specifically insensitive to oxytocin during pregnancy, or there is some obscure factor in the preparation which prevents the oxytocin from acting. It has previously been shown that the presence of œstrin in the bath has the latter effect [Marrian and Newton, 1932], and it is just possible that the traces of mucus adherent to the cervix act in the same way. The most likely explanation is the first, however, since Macdonald [1925, 1933] has shown that by

no means all smooth muscle contracts when submitted to the action of posterior pituitary extracts. In a large series of experiments [1933] he showed that 75 p.c. of renal arterial rings from various animals were inactive when exposed to even higher concentrations of the extract than those used in this work, although they could easily be made to contract or relax by other means.

Previous workers have shown that adrenaline and hypogastric stimulation act differently on the uterus in different animals, and sometimes in the same animal in different reproductive phases [summary by Gunn and Gunn, 1914]. Ergot will sometimes "reverse" the motor action, the inference being that the result of sympathetic stimulation at any one time is due to an algebraical summation of excitation and inhibition. It is therefore of interest to find, even in one animal, adrenaline causing at one and the same time contraction of one part of the uterus and relaxation of another. Fellner [1906] and Whitehouse and Featherstone [1923] differentiated between the fundus and cervix of the uterus, and came to different conclusions regarding the coordination of circular and longitudinal muscle in each of the parts, and between the two parts themselves. The latter authors state that in the rabbit hypogastric stimulation, or anæsthetization of the lumbar cord, produces contraction of the circular muscle in both parts of the organ, while stimulation of the lumbar cord causes contraction of all longitudinal muscle. They therefore postulate a sympathetic and parasympathetic innervation, there being little or no other evidence for the latter. Fleming [key reference, 1932] from physiological and careful histological investigation inclines to the view that there may be an intrinsic nervous system, of ultimate sympathetic origin, but located in the myometrium and accounting for such coordinated uterine movements as "normal" labour in the excised organ.

The present experiments show a functional coordination between the horns and the cervix, but not whether its control is vested in extra- or intra-uterine nerve cells. If labour is due to the action of an oxytocic substance, the advantages of insensitivity to such bodies on the part of the circular cervical muscle are obvious. Similarly contraction of the cervix with relaxation of the rest of the uterus would be a coordinated protective movement on the part of the organ, its initiation by adrenaline suggesting for it a sympathetic nervous origin. The question which remains unanswered is whether during pregnancy activity of the uterus is regulated by a balance between chemical and nervous mechanisms, or whether we are to look further for a nervous mechanism the opposite of

that described. If so, it will not necessarily be found in the parasympathetic, for others have shown that adrenaline may act on the same part of the uterus in different ways at different times.

Summary.

Whereas the cornua of the pregnant goat uterus are sensitive *in vitro* to a normal dose of oxytocin, the cervix is insensitive to very large doses. Adrenaline, *in vitro*, relaxes the cornua and causes the cervix to contract. The relation of these findings to the function of the uterus and to the work of other observers is discussed.

I wish to express my deep indebtedness to Prof. J. Barcroft for providing the material for these experiments, and my further thanks to him and his assistants at the Physiological Laboratory, Cambridge, for its careful selection and transmission. The remaining expenses of this research were defrayed by a grant from the Medical Research Council.

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612.144

INCREASE OF PRESSURE IN VEINS TO LEVEL OF ARTERIAL PRESSURE CAUSED BY CONSTRICTING THE LIMB IN WHICH THE VENOUS PRESSURE IS RECORDED.

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(*Received March* 1, 1934.)

The object of this paper is to prove that it is possible to raise the pressure in a limb vein to the level of arterial pressure by applying external pressure on the veins. For this purpose cannulæ were tied in the divided femoral artery and vein in opposite limbs of a dog and each was connected with a mercurial manometer. Then the arterial and venous pressures were

Fig. 1. Upper curve = arterial pressure. Lower curve = venous pressure.

recorded simultaneously. The venous pressure in the one limb was raised in the first instance by means of a piece of pressure tubing passed round the thigh proximal to the cannula in the vein, the tension of this pressure tubing being raised by rotation of a small piece of wood passed beneath it. This method of raising the venous pressure caused the rise of pressure to occur in steps on the tracing instead of a gradual rise which was desirable.

In the remainder of the experiment the vein was compressed by the inflation of a narrow rubber bag covered with linen, similar to the arm-band of a sphymomanometer. By this means the venous pressure could be raised gradually and consequently smooth tracings were obtained.

The mean arterial pressures needed correcting for the column of magnesium sulphate solution passed into the one limb of the manometer, and this was carried out as follows:

When the mean arterial pressure as measured by the manometer is 89·8 mm., the actual column of mercury displaced in one limb is one-half of this value, *i.e.* 49·9 mm., and this corresponds to the extra height of the solution of ½ sat. $MgSO_4$ sol. which has entered the manometer. This value (49·9) has to be translated into mm. of mercury thus:

$$\frac{44{\cdot}9 \times 1{\cdot}070}{13{\cdot}55} \text{ mm. Hg} = 3{\cdot}5 \text{ mm. Hg.} \qquad \tfrac{1}{2} \text{ sat. } MgSO_4 \text{ sol.}$$

Therefore the actual pressure in the artery is $88 - 3{\cdot}5 = 84{\cdot}5$ mm. Hg (1·070 being the specific gravity of the ½ sat. $MgSO_4$ sol. and 13·55 that of Hg at 20° C.).

	Actual arterial pressure in mm. Hg measured	Corrected arterial pressure in mm. Hg	Venous pressure in mm. Hg measured
Initial pressures	46·6	44·4	11·2
	89·8	86·3	70·8
	104·5	100·4	101·0
	90·25	86·7	85·5
	93·2	89·5	89·0
	96·5	92·7	93·0
	99·5	96·1	103·0
	113·0	109·1	108·4

The above figures show that the venous pressure can be raised by external compression of its vein to a height practically corresponding to the arterial pressure measured at the same time.

It will be seen that in one instance the venous pressure was higher than that of the arterial, but this was a momentary condition. Almost immediately the arterial pressure rose to a higher level than that of the veins.

Incidentally in some cases, with the raising of the venous pressure, the arterial pressure rises considerably, *e.g.* before the venous pressure was raised by compression the arterial pressure was 44·4 mm. Hg, but after compression the arterial pressure rose as high as 109·1 mm. Hg. This rise in the arterial pressure might be due to several causes, *e.g.* to stimulation of presser nerve, and if this were the case, ordinary manometric readings of human blood-pressure might be influenced in a similar way,

but there is no evidence in favour of this view. The rise might also be due to the administration of ether where in deep anæsthesia the arterial pressure falls and rises with light anæsthesia. We are convinced that the latter is the explanation of the arterial rise in this case.

Zarday and Zilahy [1931] have made similar experiments with similar results.

We are greatly indebted to Prof. Macdonald for the interest he took in the experiment and for permission to use the laboratory and material.

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612.45:612.893

THE MECHANISM OF THE NERVOUS DISCHARGE OF ADRENALINE.

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(*Received March* 12, 1934.)

Recently two of us [W. F. and B. M., 1933] have shown that a substance pharmacologically indistinguishable from acetylcholine can be detected in the venous blood collected from the suprarenals during splanchnic stimulation, when eserine is present, but that no such substance is present in the absence of stimulation. It seemed to us that it should be possible to demonstrate the effects of this liberated acetylcholine either on the arterial blood-pressure or on a distant organ of the same animal, if the blood from the suprarenals containing it were allowed to flow normally into the general circulation. This possibility was tested in the experiments described in the first part of this paper. To detect the acetylcholine by its action on a distant organ we used the submaxillary gland.

Experiments thus to detect acetylcholine, by its action on other systems of the animal into the blood of which it is liberated, are complicated by the actions of adrenaline, the output of which from the medullary cells is the main and more obvious effect of the splanchnic stimulation. It is necessary in some way to suppress the actions of this adrenaline, or, allowing it to produce its normal effects, to demonstrate the action of acetylcholine only as a complication or phase of the total effect. Both methods have been used.

The demonstration that acetylcholine is liberated from the suprarenal bodies during splanchnic stimulation does not, however, provide a direct proof that acetylcholine is the direct cause of the adrenaline discharge. As evidence of this function of acetylcholine, we consider, in the second part of this paper, the effect of eserine on the response of the suprarenals to splanchnic stimulation by the discharge of adrenaline.

[1] The experiments here recorded were begun in the Physiological Institute of the University of Berlin. When they were there interrupted, one of us (W.F.) had the privilege of continuing and completing the work at the National Institute for Medical Research.

Further experiments, which are described in the third part of the paper, deal with the effects of nicotine and atropine on the actions of acetylcholine and of splanchnic stimulation on the suprarenals.

It will be seen that, in addition to the predominant "nicotine" action of acetylcholine on the medullary cells, there is evidence of a subsidiary "muscarine" action, surviving nicotine paralysis and requiring atropine for its suppression. In the same connection we have taken the opportunity of examining the action of certain other alkaloids on the medullary cells.

Methods.

The experiments were made on cats under chloralose anæsthesia or on spinal cats. In the latter the preliminary operation under ether was performed by removing the roof of the second cervical vertebra, cutting the cord and destroying the brain through the foramen magnum. In some experiments the whole spinal cord was destroyed as well.

For the stimulation of the splanchnic fibres to the suprarenal bodies, the stomach, spleen, small and large intestine were removed, and the vessels of the kidneys were tied in addition. The splanchnics were cut in the chest, small incisions being made through the diaphragm. They were tied and dissected for stimulation. Artificial respiration was used in all these experiments. It has proved convenient to remove part of the liver, especially of the left lobe, in order to obtain more room for the electrodes. This was especially useful in the experiments in which the left splanchnic was stimulated repeatedly with fluid electrodes, which had to be fixed in position. The stimulation with fluid electrodes was only carried out when the effect of repeated stimulations had to be compared quantitatively; otherwise ordinary metal electrodes were used. In the earlier experiments fluid electrodes of the type described by Keith Lucas, later those of Brown and Garry [1932] as modified by Collison [1933] were used.

In the experiments in which the direct secretory action of drugs on the medulla of the suprarenals was examined, we adopted a method in which small amounts of the drug were injected into the abdominal aorta above the suprarenals. For this purpose the cat was eviscerated as above, the renal vessels were tied near the kidneys, the abdominal aorta and vena cava were tied below the suprarenals, and a cannula was tied into the central end of the clamped cœlic artery. Through this cannula the injections were made. In the earlier experiments we used an ordinary fine glass cannula, the free end of which was connected and closed with a rubber tube. The injections were made through the rubber tube in the same way as ordinary intravenous injections. Later we used the special

cannula for arterial injections described by Chang and Gaddum [1933]. If a drug is injected in this way it reaches the suprarenals with relatively little dilution by the blood. An action on the suprarenals can therefore be obtained with amounts of the drug so small that, with intravenous injection, they would cause little or no discharge of adrenaline. This kind of injection has shown itself to be especially suitable if used for drugs with strong depressor action [Feldberg and Minz, 1931; Szczygielski, 1932; Gutmann, 1932; Feldberg, 1932; Siehe, 1934]. These drugs injected intravenously cause only a fall of pressure, by which their actions on the suprarenals are completely masked; whereas the effect of the same drug injected arterially, in the same or even in smaller doses, may be mainly pressor, owing to the discharge of adrenaline. By comparing the effects of venous and arterial injections, the secretory action of a drug on the suprarenals can thus be demonstrated. For the intravenous injections a cannula was tied into the jugular vein. For complete evidence of action on the suprarenal medulla, it is necessary to show, further, that the pressor action seen with arterial injection is abolished when both suprarenals are removed.

Experimental.

(i) *Direct evidence of liberation of acetylcholine.*

(*a*) *The effect on the arterial blood-pressure.* In an eviscerated cat, the pressor response of the arterial blood-pressure to stimulation of the splanchnic nerves is caused only by the output of adrenaline from the suprarenals. If the destruction of acetylcholine is inhibited by a previous intravenous injection of eserine, this pressor response is modified in different but characteristic ways. The rise of pressure may then be delayed, or interrupted in its early stages, or preceded by a fall of pressure. Further, if the blood-pressure of the cat has been made insensitive to adrenaline itself, as during the persistent effect of an earlier supramaximal injection of a few mg. of adrenaline, we obtain only the depressor action of acetylcholine as the result of stimulating the splanchnic nerves. These effects of the liberated acetylcholine are all abolished by atropine.

(1) On eviscerated cats with very low blood-pressure the rise of pressure caused by splanchnic stimulation is often delayed after the injection of eserine. This effect is shown in Fig. 1. In this and other figures the beginning and end of stimulation are recorded not only by the base-line signal but by vertical lines intersecting the blood-pressure curve itself, produced by rapid compression and release of the rubber

tubing leading to the manometer. The splanchnic nerves were stimulated at I and II, for 30 sec. in each case, with the secondary coil at 10 cm. Between I and II, 6 min. before II, 0·3 mg. of eserine was injected intravenously. At the first stimulation the blood-pressure rose during the stimulation, the rise starting 21 sec. after the onset. At the second stimulation the blood-pressure did not rise until 6 sec. after the stimu-

Fig. 1. Carotid blood-pressure of a cat (also in all other figures). Spinal eviscerated. I and II stimulation of left splanchnic, 30 sec., coil at 10 cm. Between I and II 0·3 mg. eserine.

Fig. 2. Chloralose. Eviscerated. At I, II, III and IV stimulation of the left splanchnic, 10 sec., coil at 8 cm. Between I and II 0·4 mg. eserine, between III and IV 0·3 mg. atropine intravenously.

lation had ceased, or 36 sec. after its onset. The pressor response was therefore delayed by 15 sec. In other experiments the delay after eserine was not quite so long, being between 7 and 12 sec. After an intravenous injection of 0·1 mg. of atropine the delay produced by eserine was abolished.

The delay is chiefly, but not wholly, due to the action of the acetylcholine liberated during the nerve stimulation and counteracting the

pressor effect of adrenaline. A similar delay in the pressor response occurs if a small amount of acetylcholine is mixed with adrenaline and the two drugs are injected together [Feldberg and Minz, 1933]. A part of the delay, however, is due to the slowing of the circulation which occurs after eserine, which causes the liberated adrenaline to be carried to the arteries more slowly than before the eserine injection. Thus the pressor response to an intravenous injection of adrenaline showed also a delay after eserine; but this amounted only to 2–4 sec., compared with a delay of the splanchnic response, in the same experiments, lasting 12–15 sec.

(2) If the blood-pressure is not quite so low, or if the acetylcholine can act more vigorously, the rise caused by splanchnic stimulation will be interrupted in its early stages. This is shown by the experiment given in Fig. 2, which is also taken from an eviscerated spinal cat. At I, II, III and IV the left splanchnic was stimulated for 10 sec., with the secondary coil at 8 cm. The effect of the first stimulation (I) was an uninterrupted rise of pressure, which began as soon as the stimulation had stopped. After this the cat was given 0·4 mg. of eserine intravenously, which caused a slight slowing of the heart beat. Five and 15 min. later the second (II) and third (III) stimulations were made. The blood-pressure began to rise, in both cases, as soon as the stimulation had ceased, but after a few seconds the rise was interrupted by a slight transitory fall, after which the main secondary rise set in. This began about 12 sec. after the primary rise. In other experiments all types of result between a simple delay and an actual interruption of the rise were seen. Being due to the action of the acetylcholine, the fall is abolished by atropine: 0·1 mg. of atropine was given intravenously 3 min. before Fig. 2, IV. A similar interruption of a pressor response is sometimes obtained if adrenaline is injected intravenously, mixed with acetylcholine.

(3) On eviscerated, spinal cats with high blood-pressure the pressor response to splanchnic stimulation is sometimes preceded by a pronounced fall of pressure, if eserine has been injected beforehand. This fall is also due to the action of the acetylcholine liberated during the stimulation of the nerve, and is abolished by atropine. These results are more easily obtained on eviscerated cats under chloralose having a rather high blood-pressure. Under these conditions, however, adrenaline itself in small doses may have a depressor action, so that the adrenaline discharged during splanchnic stimulation may by itself produce a fall of pressure followed by a rise. This fall, however, is not abolished by

atropine and is seen before the injection of eserine. It can, therefore, easily be distinguished from the acetylcholine effect, which only occurs after eserine and is abolished by atropine. If we obtain a fall of pressure during splanchnic stimulation before the injection of eserine, due to the depressor action of the commencing discharge of adrenaline itself, the fall is greatly deepened after eserine; and this augmentation of the depressor effect of the splanchnic stimulation is abolished by atropine, and may accordingly be attributed to acetylcholine.

Fig. 3. Chloralose. Eviscerated. At I, II and III stimulation of left splanchnic, 15 sec., coil at 6 cm. Between I and II 0·5 mg. of eserine intravenously. Level and position of drum adjusted. Between II and III 0·2 mg. atropine.

In several cats under chloralose, however, adrenaline, as well as splanchnic stimulation, caused only a rise of pressure. If eserine was injected into these animals, splanchnic stimulation then caused a strong fall followed by a rise. The depressor action was then abolished by atropine. This is shown by the record given in Fig. 3. At I, and II and III the left splanchnic is stimulated for 15 sec. with the secondary coil at 6 cm. The stimulations are made 8 min. from each other. Between I and II 0·5 mg. eserine, between II and III 0·1 mg. atropine was injected intravenously. We see that the simple rise in I is preceded by a fall in II, which is again absent after atropine at III.

In the experiments giving Figs. 1 and 3 we see, further, that the

pressor response to splanchnic stimulation is greater after the injection of eserine, whereas it is smaller in Fig. 2. We cannot conclude definitely that these changes were due to the eserine, though it is possible. The nerves were stimulated in these experiments with ordinary metal electrodes, so that the effects of the different stimulations, in each experiment, may not be strictly comparable. On the other hand we might expect, on different occasions, either an increased or a decreased pressor effect after eserine. Eserine causes an increased output of adrenaline from splanchnic stimulation, as we shall see later, which will tend to increase the pressor response. On the other hand, acetylcholine liberated during the stimulation is not destroyed after eserine. According as the

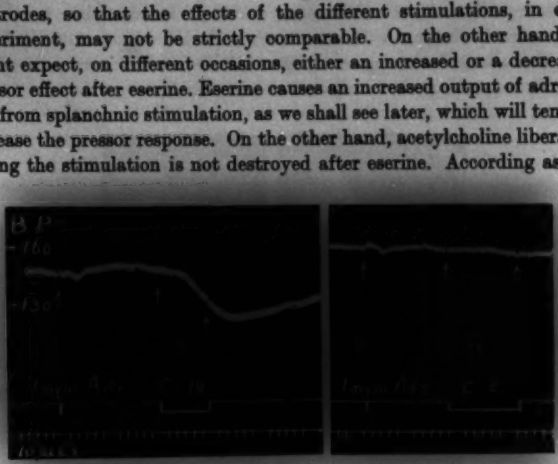

Fig. 4. Chloralose. Eviscerated. Arterial blood-pressure insensitive to adrenaline from previous injections of 4·1 mg. in all. 20 min. before I 0·8 mg. eserine intravenously. At I and III 1 mg. of adrenaline; at II and IV stimulation of both splanchnic nerves, coil at 10 and 8 cm. Between II and III 0·1 mg. of atropine.

increased output of adrenaline or the counteracting effect of the liberated acetylcholine prevails, we may therefore obtain a stronger or weaker pressor response.

(4) Fröhlich and Pick [1913] have shown that the blood-pressure of a cat will become insensitive to further injection of adrenaline after a few mg. have been injected intravenously. These results were confirmed by one of us [W. F.] in this laboratory six years ago. Under these conditions, however, acetylcholine will still produce its depressor action, though its effect is weakened. In this state of insensitiveness to adrenaline, stimulation of the splanchnic ought to result only in a fall of blood-pressure, if the destruction of the liberated acetylcholine is inhibited by a previous injection of eserine. This is demonstrated by the experiment illustrated by Fig. 4.

The cat (3·2 kg.) was anæsthetized with chloralose and eviscerated. It received first an intravenous injection of 0·1 mg. adrenaline. During the rise which followed the injection, 1 mg. adrenaline was injected and produced a further rise of pressure. A further injection of 1 mg. however, given 1 min. later, was already ineffective. The injection was repeated twice with the same result. Both splanchnic nerves were then stimulated for 1 min. with the secondary coil at 10 cm. The stimulation had no effect on the arterial blood-pressure. The cat was then given 0·8 mg. eserine intravenously, which caused a slight, gradual fall of pressure. A new intravenous injection of 1 mg. adrenaline was still ineffective. This injection is shown in Fig. 4, I. Two minutes later both splanchnics were stimulated for about 80 sec. with the secondary coil at 10 cm. The stimulation resulted in a prolonged fall of pressure, which started about 40 sec. after the beginning of the stimulation (II). The cat then received an intravenous injection of 0·1 mg. atropine, after which the blood-pressure rose again. A further injection of 1 mg. of adrenaline was still without effect (III), and renewed stimulation of both splanchnics for 100 sec. with the coil at 8 cm. caused neither fall nor rise of pressure (IV).

(*b*) *The effect on the submaxillary gland.* To demonstrate the sialogogue effect of the acetylcholine liberated during splanchnic stimulation from the suprarenals of a cat, one must exclude the effect of the discharged adrenaline, which would itself produce a secretion of saliva. This can be done by injecting intravenously a few mg. of ergotoxine. The salivary glands poisoned by ergotoxine no longer react to adrenaline, but still respond to chorda stimulation or acetylcholine. If eserine has also been injected, the acetylcholine, which is now protected from esterase and liberated into the blood stream during the stimulation, ought to cause a secretion of saliva: and this secretory effect ought to be abolished again by atropine.

These expectations were realized experimentally, and are demonstrated by the experiment illustrated in Fig. 5, which is taken from a 3 kg. cat in chloralose anæsthesia. The stomach, spleen, small and large intestine of the cat were removed, and the circulation was further restricted by tying the aorta and vena cava below the suprarenals. A cannula was tied into the right Wharton's duct and connected with a long horizontal glass tube with millimetre scale. The chorda-lingual nerve was cut on the same side. An intravenous injection of 0·1 mg. adrenaline caused a secretion of 0·8 c.c. of saliva, and stimulation of both splanchnics produced also a slight secretion of the salivary glands at that stage. These

effects, however, were completely abolished after 3 mg. ergotoxine had been injected intravenously, showing that the salivary glands had become insensitive to adrenaline. The cat then received two intravenous injections of 0·9 mg. eserine, which were given 25 min. apart from each other. Thereafter both splanchnics were stimulated anew, for 1 min., with the secondary coil at 7·5 cm. This stimulation resulted in a long-lasting secretion of saliva as shown in Fig. 5. The secretion started about

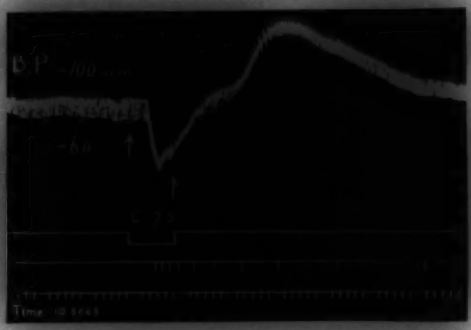

Fig. 5. Arterial blood-pressure (upper tracing) and record of salivary secretion (middle tracing). Chloralose. Evisceration and restriction of circulation. 1·8 mg. eserine and 3 mg. ergotoxine given earlier. Stimulation of both splanchnic nerves, coil at 7·5 cm.

half a minute after the beginning of the stimulation and amounted to 0·5 c.c. of saliva, each mark in Fig. 5 corresponding to the secretion of 0·05 c.c. This secretion cannot be due to the effect of the discharged adrenaline, because an intravenous injection of 0·1 mg. adrenaline, given a few minutes after the stimulation, was ineffective, showing that the salivary glands were still completely insensitive to adrenaline. The secretion must be due to the acetylcholine liberated from the suprarenals during the stimulation and protected from destruction by the eserine. This effect, accordingly, was absent when the stimulation was repeated after an injection of 0·1 mg. atropine. This is a further proof that it cannot be due to the discharge of adrenaline, the secretory effect of which is not affected by atropine. The fall of arterial pressure in Fig. 5 may also be partly due to the acetylcholine liberated, but is due in part to the reversed action, after ergotoxine, of adrenaline.

(ii) *Acetylcholine as the stimulus for adrenaline output.*

If the output of adrenaline from the suprarenals, caused by splanchnic stimulation, is actually due to the liberation of acetylcholine to act as the direct stimulant of the medullary cells, eserine would be expected to increase the effect. Similarly eserine ought to increase the effect of acetylcholine artificially injected, in causing an output of adrenaline from the glands. Even this latter effect of eserine, however, has not hitherto been proved. There is only the statement of White and Stedman [1931] that the pressor action of an intravenous injection of acetylcholine, in atropinized cats, is increased by a previous injection of miotine, a synthetic urethane compound, which has an eserine-like action; but the pressor response of acetylcholine is only in part attributable to the effect of acetylcholine on the suprarenals. It seemed, therefore, necessary to determine whether the secretory effect of acetylcholine on the suprarenal bodies is actually increased by eserine. As controls for these experiments with acetylcholine, we have tested the effect of other drugs, such as choline and histamine, which also cause secretion from the medulla of the suprarenals.

It was necessary first to be certain that the effect of adrenaline itself is not sensitized by eserine. Intravenous injections of the same dose of adrenaline, given before and after an intravenous injection of eserine, showed no differences in the pressor responses. Any change that might occur after eserine in the pressor effects of histamine, choline, acetylcholine or splanchnic stimulation on the suprarenals, must therefore be due to alterations in the output of adrenaline, and not to changes in the effects of adrenaline itself.

(*a*) *The effect of eserine on the adrenaline discharge caused by acetylcholine.* If acetylcholine is injected through the cannula tied into the central end of the cœliac artery of an eviscerated cat, the circulation of which has been restricted (see "Methods"), its pressor effect is wholly due to its secretory action on the suprarenals. This pressor response is greatly enhanced by eserine, as is shown by the experiment given in Fig. 6 from a cat under atropine. Injections of 0·02 mg. acetylcholine were repeated several times before eserine with constant effect. The last two are shown in Fig. 6, *A* and *B*. Between *B* and *C*, 1 mg. eserine was given intravenously. Five and 25 min. later 0·02 mg. acetylcholine was injected again. It can be seen that the pressor responses to these injections are greater than those before the eserine injection. The pressure rises higher and the effect lasts longer. At *E* the injection of acetylcholine was

repeated after removal of the suprarenals. The lack of any pressor response shows that those caused by the previous injections of acetylcholine were wholly due to excretory effects on the suprarenals.

Under the same conditions we have studied the effects of choline and histamine separately on the suprarenals, and have found that eserine produced no increase of the pressor responses to these substances.

(*b*) *The effect of eserine on the adrenaline discharge caused by splanchnic stimulation.* These experiments have also been carried out on cats under chloralose or with the brain destroyed. The cats were eviscerated, the vessels of the kidneys were tied, atropine was injected intravenously before stimulation, and the left splanchnic was stimulated with fluid electrodes. Under these conditions repeated stimulations, for equal

Fig. 6. Chloralose. Eviscerated. Renal vessels, abdominal aorta and vena cava tied below the suprarenals. 0·2 mg. atropine intravenously. At *A*, *B*, *C*, *D*, and *E* arterial (coeliac) injections of 0·02 mg. of acetylcholine. Between *C* and *D* 1 mg. eserine intravenously. Between *D* and *E* both suprarenals removed.

lengths of time and with the same strength of current, gave practically identical pressor responses, wholly due to the output of adrenaline from the suprarenals. These pressor responses were increased after an injection of eserine. In some experiments the increased effect was chiefly revealed by the higher maximum, in others by the greater persistence of the pressor effect. These results are demonstrated in Fig. 7, from a cat under chloralose. Before the stimulation 0·2 mg. atropine was injected intravenously. At I, II, III and IV the left splanchnic was stimulated, for 20 sec. in each case, with the secondary coil at 12 cm. Between II and III 1 mg. eserine, and between III and IV a further 0·2 mg. atropine was injected. After eserine the blood-pressure rises higher and stays much longer at the high level than at I and II. Further, instead of the small interruption which takes place at I and II in the early stages of the rises,

we obtain in III and IV a strong fall preceding the rise of pressure. This must be interpreted as also due to an increased output of adrenaline, since the atropine excludes its production by acetylcholine. A similar depressor effect, followed by a rise of pressure, was also obtained before the injection of eserine, when the splanchnic nerve was stimulated with the coil at 9 cm. instead of 12 cm. The injection of atropine was repeated after III, but the depressor action persisted at IV. The depressor effect is therefore, in this case, due to the commencing output of adrenaline.

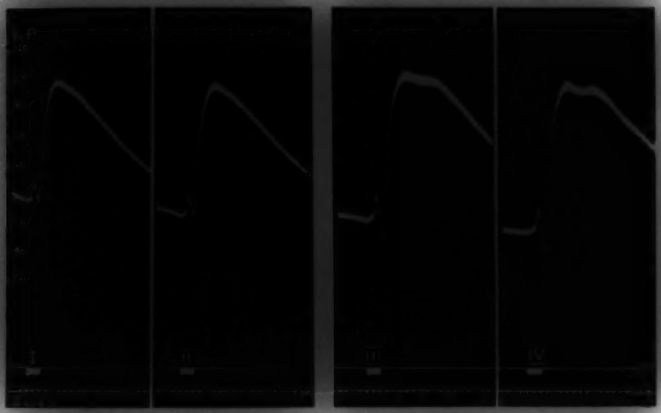

Fig. 7. Chloralose. Eviscerated, renal vessels tied. 0·2 mg. atropine intravenously. At I, II, III and IV stimulation of left splanchnic (fluid electrodes) for 20 sec., coil at 12 cm. Between II and III 1 mg. eserine intravenously. Between III and IV 0·2 mg. atropine intravenously.

The effect of stimulation with the coil at 12 cm. being much increased by eserine, we moved the secondary coil outwards till an effect was obtained, at a distance of 18 cm., approximately equal to that obtained before eserine with the coil at 12 cm.

In order to show that eserine has generally no sensitizing action on sympathetic nerve effects, we examined its effect on the vaso-constriction caused by stimulating sympathetic nerves. We stimulated either the abdominal sympathetic chains on eviscerated cats, or the left splanchnic nerve after removal of the left suprarenal body, with the viscera intact. The vaso-constriction produced by the abdominal sympathetic chains

resulted only in a slight rise of arterial blood-pressure, due to vaso-constriction chiefly in the hindlegs, whereas stimulation of the left splanchnic, after removal of the left suprarenal body, caused a large rise of pressure, due to vaso-constriction in the abdominal viscera. These stimulations were all carried out with fluid electrodes, and practically identical pressor responses could be obtained if the stimulations were repeated with the same strength of current and the same duration. If an intravenous injection of 0·5–1 mg. of eserine was given between two such stimulations, the pressor responses were usually unaffected. In a few experiments only, in which the left splanchnic was stimulated, the fall of the pressure to the original level, after the stimulation, seemed to be slower when eserine was present. This effect, however, was not constant and was not very marked, compared with the strong sensitization of the adrenaline discharge. On the other hand, it must be remembered that, in stimulating the splanchnic nerves, we are dealing with preganglionic vaso-constrictor fibres, and from the experiments of Feldberg and Gaddum [1933] we can assume that the impulses passing from preganglionic to ganglion cells are transmitted by acetylcholine. The slight sensitization observed occasionally may, therefore, be due to an effect of eserine on the ganglion.

We may here draw attention to an observation which, though it has no relation to our present enquiry, may have some significance not yet clear. Much weaker currents (secondary coil at 20–24 cm.) were sufficient for stimulating the directly vaso-constrictor fibres in the splanchnic than were required for its secretory effect on the suprarenals (secondary coil at 8–12 cm.).

(iii) *"Muscarine" and "nicotine" actions on the suprarenal medulla.*

The expression "muscarine-" and "nicotine-like actions" was first used by Dale [1914] in his study of the pharmacological action of choline and its derivatives. He found that the depressor action of these drugs on the arterial blood-pressure was completely abolished by small doses of atropine, whereas the pressor action which then remained, and which was assumed to be chiefly due to an action on the sympathetic ganglia, and the suprarenal medulla, was completely abolished by nicotine. This distinction, which is based on the sensitiveness of the effect to atropine and nicotine, has been generally accepted. One must take into account, however, the fact that even the nicotine-like action can be abolished if very large doses of atropine are used [Feldberg and Minz, 1931]. On the other hand, it seems that very large doses of nicotine have some

weakening effect on the muscarine-like action. The distinction between the muscarine-like and nicotine-like actions is, therefore, not absolutely sharp, but if the overlapping effects of very large doses of atropine and nicotine are excluded, it holds true. As the nicotine-like action of splanchnic stimulation and acetylcholine are well known, the following experiments are especially concerned with the muscarine-like action of these two, *i.e.* with that part of the effect, which is sensitive to small doses of atropine.

(*a*) *Splanchnic stimulation.* The "muscarine-like" effect of splanchnic stimulation on the suprarenal medulla cannot so easily be demonstrated. If we compare the rise of pressure caused in an eviscerated spinal cat, by stimulating the left splanchnic with fluid electrodes before and after an intravenous injection of a small dose of atropine, no very convincing reduction of the pressor effect will be seen.

Even after large doses of nicotine, which almost completely abolish the effect of splanchnic stimulation on the suprarenal medulla, the remaining effect will only be slightly but definitely reduced further by an intravenous injection of atropine. It seems as if the large doses of nicotine which are necessary to abolish the nicotine-like action also affect the slight muscarine-like effect. The best way of demonstrating the existence of a muscarine component is as follows. In an eviscerated cat large doses of nicotine are first injected, till the pressor response to splanchnic stimulation is almost completely abolished. If eserine is then injected in addition the surviving remnant of the pressor response is greatly enhanced. This effect, however, is then completely abolished by a small dose of atropine. Fig. 8 is a record from such an experiment. The tracing is taken from an eviscerated spinal cat. The strong pressor response, which was obtained at the beginning of the experiment by stimulating the left splanchnic nerve with fluid electrodes for 20 sec., with the secondary coil at 8·5 cm., was nearly completely abolished by five previous intravenous injections each of 5 mg. nicotine. Only the slight effect seen at Fig. 8 remained: 0·8 mg. of eserine was then injected, and a few minutes later the left splanchnic was stimulated again, at II, with the same strength of current and for the same length of time, and now produced a strong pressor response. This was not reduced, if 5 mg. of nicotine were again injected intravenously. If, however, a small dose of atropine (0·1 mg.) was injected, the stimulation became ineffective (Fig. 8, III).

(*b*) *Acetylcholine.* The experiment illustrated in Fig. 9 demonstrates the "muscarine" action of acetylcholine on the suprarenal medulla.

Small doses of nicotine and acetylcholine were given to the glands by arterial injection, using the method already described. These doses were

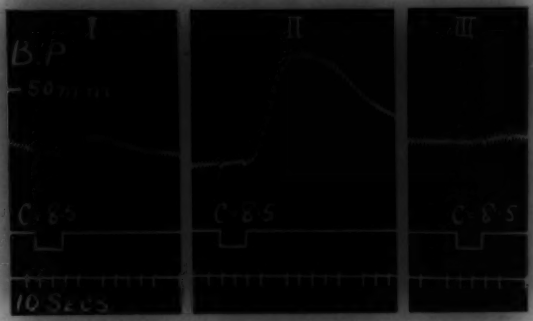

Fig. 8. Chloralose. Eviscerated. At I, II and III stimulation of left splanchnic (fluid electrodes), 20 sec., coil at 8·5 cm. Cat had previously received 25 mg. nicotine in all intravenously. Between I and II 0·8 mg. eserine intravenously, between II and III 0·1 mg. atropine intravenously.

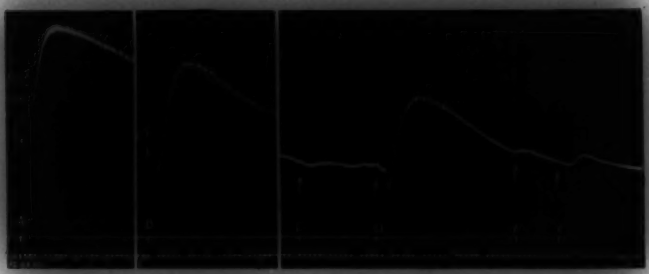

Fig. 9. Chloralose. Artificial respiration, eviscerated. Renal vessels, abdominal aorta and vena cava tied below suprarenals. At *A* and *C* arterial (cœliac) injection of 0·1 mg. nicotine; at *B*, *D* and *F* arterial injection of 0·02 mg. acetylcholine; between *B* and *C* 12 mg. nicotine in all intravenously. At *E* 0·05 mg. atropine intravenously.

chosen so as to give approximately equal outputs of adrenaline, as shown by the pressor responses. The comparison could not be made with great accuracy, because the pressor effect of adrenaline liberated in response to acetylcholine was complicated by preliminary depressor effects, due to

traces of acetylcholine escaping into the general circulation, whereas nicotine similarly escaping would have an additional pressor action. Subject to such corrections, it may be said that the output of adrenaline produced by 0·1 mg. nicotine, at Fig. 9, *A*, is of the same order as that produced by 0·02 mg. of acetylcholine at *B*. Between *B* and *C* 12 mg. of nicotine were given intravenously. This sufficed to annul the effect of a further 0·1 mg. nicotine injected arterially at *C*. It may be noted that even much larger doses of nicotine would not suppress the effect of larger (3–5 mg.) doses of that alkaloid, given arterially, on the suprarenal medulla. The point of interest for our present purpose, however, is that

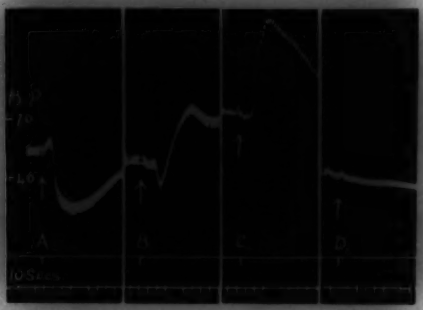

Fig. 10. As in Fig. 9. At *A* intravenous, at *B*, *C* and *D* arterial (coeliac) injection of 0·1 mg. arecoline. Between *B* and *C* 0·08 mg. atropine. Between *C* and *D* 15 mg. nicotine intravenously.

the effect of 0·1 mg. was annulled (*C*) by treatment which left a substantial part of the action on the suprarenal medulla of 0·02 mg. of acetylcholine (at *D*)—a dose which had earlier been roughly equivalent in action to 0·1 mg. nicotine. An injection of 0·1mg. of atropine, at *E*, now suppresses almost the whole of the action of 0·02 mg. of acetylcholine, given again at *F*, which had survived the nicotine paralysis. Nearly the whole of the action at *D*, therefore, must have been of the "muscarine" type.

(*c*) *Muscarine.* A few experiments were tried with arterial injections of true muscarine. This alkaloid, however, is so stable, and its depressant effects on the heart and general circulation were so prolonged, that it was difficult to be certain of its action on the suprarenal medulla. In several cases, however, we have recorded an undoubted, though weak,

pressor effect, beginning about 30 sec. after the arterial injection, and, like the direct effects of muscarine, abolished by atropine. The observation only has interest in showing that a pure muscarine action, without any suspicion of a "nicotine" component, does stimulate to some extent the output of adrenaline from the medullary cells.

(*d*) *Pilocarpine.* Dale and Laidlaw [1912] have shown that pilocarpine causes a secretion of adrenaline from the suprarenals. They, however, did not study the effect more in detail. We found that the effect is a peripheral one and that it is completely abolished by a small dose of atropine. This effect appears, therefore, to be of the muscarine type, and is not as strong as that of acetylcholine.

(*e*) *Arecoline.* Arecoline, of which hitherto only parasympathomimetic effects have been described, has a strong secretory action on the suprarenal medulla. This effect is stronger than that produced by pilocarpine, but weaker than that produced by acetylcholine. It differs from the secretory action of muscarine and pilocarpine on the suprarenals in that it is resistant to small doses of atropine (Fig. 10). The action of arecoline on the suprarenal medulla is, therefore, chiefly, if not wholly, of the nicotine type. This observation corresponds with more recent ones, to be published later, on the action of arecoline on ganglion cells.

Discussion.

The demonstration that acetylcholine is liberated from the suprarenal glands during splanchnic stimulation does not, by itself, prove that it is actually the direct stimulant of the medullary cells to secrete adrenaline. The liberation of acetylcholine might be an incidental effect of the nerve stimulation; it might not even be liberated in relation to the medullary cells at all, but in the cortex of the gland. This possibility reminds us of the old conception of Lohmann [1907], now obsolete, that choline is the hormone of the cortex, which thus would liberate a hormone acting like parasympathetic nerve stimulation, and antagonistic to adrenaline, the "sympathetic" hormone of the medulla. This theory was based on the observation that the cortex contained a large amount of choline, whereas the medulla contained very little. Recent observations, as yet unpublished, by H. Schild and one of us [W. F.], however, have shown that the concentration of acetylcholine, or of a substance pharmacologically indistinguishable from it, is twice to four times as high in the medulla as in the cortex. This points strongly in the direction of the medulla as the seat of the acetylcholine liberation. The experiments of

the present paper, in which the influence of eserine on the adrenaline discharge caused by splanchnic stimulation is shown, provide additional evidence for the view that acetylcholine is the direct stimulant of the medullary cells transmitting the effect of the nerve stimulation. After eserine the acetylcholine liberated would not be destroyed so easily, and should therefore be able to act longer and more vigorously. We have shown that the adrenaline discharge caused by splanchnic stimulation is, in fact, increased by eserine.

A more detailed analysis of the action of acetylcholine and splanchnic stimulation on the suprarenal medulla showed that the action in both is due mainly to the "nicotine" aspect of its action, which is abolished by large doses of nicotine, but partly also to a "muscarine" action, which is abolished by small doses of atropine. This parallelism of a double action, in the case of acetylcholine and of splanchnic stimulation, is a further indication that the adrenaline discharge is due to the same direct stimulus in both cases.

All our experiments, therefore, point in the same direction. They show that the impulses travelling down the splanchnic nerve fibres to the suprarenal medulla are chemically transmitted to the effector cells, by liberation of something indistinguishable from acetylcholine.

Hitherto acetylcholine has been regarded as the humoral transmitter of parasympathetic effects only. The splanchnic fibres to the suprarenals are sympathetic. The fact that impulses travelling down these fibres are also chemically transmitted by acetylcholine provides a first example of the transmission of preganglionic impulses by liberation of that substance. A further example, of more general significance, is presented in the adjoining paper by Feldberg and Gaddum.

Summary.

1. The liberation of acetylcholine from the suprarenals during splanchnic stimulation is shown by its effect on the arterial blood-pressure and the salivary glands of the same animal.

2. The adrenaline discharge caused by splanchnic stimulation is greatly enhanced by eserine.

3. The action of acetylcholine as well as of splanchnic stimulation on the suprarenal medulla is mainly a "nicotine" action, which can be abolished by larger doses of nicotine; it has also, however, a "muscarine" component, which is abolished by small doses of atropine.

4. It is concluded that acetylcholine is the humoral transmitter of

splanchnic impulses to the suprarenal medulla, so that the preganglionic sympathetic fibres are cholinergic.

5. Muscarine and pilocarpine cause an output of adrenaline from the suprarenals. These effects are completely abolished by atropine. Arecoline also causes a discharge of adrenaline, which is wholly or mainly resistant to atropine, but abolished by nicotine.

We desire to make grateful acknowledgment of our debt to the Medical Research Council for hospitality and to Sir Henry Dale for help and advice.

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612.89:547.435–292

THE CHEMICAL TRANSMITTER AT SYNAPSES IN A SYMPATHETIC GANGLION.

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(*Received March* 12, 1934.)

It was shown by Dale [1914] that acetylcholine and other choline esters have two types of action:

(1) "Muscarine" actions, paralysed by atropine, and reproducing the peripheral effects of stimulating parasympathetic nerves.

(2) "Nicotine" actions, of which the most conspicuous is the stimulant action on the cells of sympathetic ganglia, and probably of all autonomic ganglia. These "nicotine" actions are unaffected by atropine, but are paralysed by large doses of nicotine.

The correspondence between the "muscarine" actions of choline esters and peripheral parasympathetic effects has found its explanation in the evidence, rapidly accumulating since Loewi's first demonstration on the frog's heart vagus [1921], that parasympathetic nerves produce their effects by peripheral release of a choline ester, having properties similar to those of acetylcholine; that, in the terminology suggested by Dale [1933], postganglionic parasympathetic fibres are "cholinergic." Since acetylcholine itself occurs in the animal body, and is the only ester of choline known to do so, it is generally accepted as the probable chemical transmitter concerned. Till recently there was no evidence that the "nicotine" action of acetylcholine, or of any choline derivative, might also be concerned in the chemical transmission of nervous effects. In the course of a study of the distribution of choline esters, and of acetylcholine in particular, in the animal body, Chang and Gaddum [1933] found that extracts of the sympathetic chain of a horse contained comparatively large amounts of a substance having the physiological properties of acetylcholine, and were led to suggest that this substance might play a part in the normal transmission of impulses through the ganglia, having

in view its stimulant action on ganglion cells. The first direct evidence, however, for such a cholinergic function of preganglionic sympathetic fibres was obtained in experiments by Feldberg and Minz [1933] and Feldberg, Minz and Tsudzimura [1933] on the secretion of adrenaline from the suprarenal medulla in response to splanchnic stimulation.

The experiments here described have furnished evidence that the preganglionic fibres ending in relation to the nerve cells of a sympathetic ganglion are also cholinergic. The method used has been essentially one described by Kibjakow [1933], who perfused the superior cervical ganglion in an anæsthetized cat with oxygenated Locke's solution, and recorded the contractions of the nictitating membrane, which, under these conditions, were still evoked by stimulation of the cervical sympathetic nerve. Kibjakow collected the Locke's solution flowing from the vein of the perfused ganglion, and reinjected it into the fluid flowing to the artery of another, or in some cases of the same perfused ganglion. He found that such reinjection of the venous fluid collected during strong stimulation of the cervical sympathetic nerve caused contraction of the nictitating membrane, comparable to that caused directly by nerve stimulation, but that fluid flowing from an unstimulated ganglion had no such action. He regarded this as evidence of chemical transmission in the ganglion, and Chang and Gaddum suggested that the substance concerned might be acetylcholine. It will be seen that our experiments give definite evidence of the liberation of acetylcholine under such conditions, though we have not yet been able to reproduce the reinjection effect, described by Kibjakow, with any regularity.

Methods.

Cats were anæsthetized with chloralose and the ganglion prepared for perfusion by Kibjakow's method. The upper part of the carotid artery was exposed. A transverse vein was divided between ligatures and a lymphatic gland removed. The carotid artery was doubly ligatured and divided beyond the point where the artery to the ganglion arises. All the arterial branches except those running to the tissue round the ganglion were tied and divided. The small internal carotid artery was separated from the postganglionic sympathetic branches and divided. Numerous small veins were also divided, leaving only the vein from which the perfusion fluid was to be collected, with the small vein from the ganglion opening into it. The most convenient vein for this purpose is the internal

jugular. When this vein was too small, as it often was, the fluid was collected from the vein which emerges from the vertebral column through the atlantal foramen, and joins the jugular vein near the ganglion; the jugular vein being, in that case, tied above and below the entry of the vein from the ganglion.

Suitable lengths of the cervical sympathetic nerve and the common carotid artery were freed and the cat's head was then fixed in position, by a clamp holding the upper jaw, so that the contraction of the nictitating membrane could be recorded, by attaching its free edge, by means of a cotton thread passing round a pulley, to a lever. "Chlorazol fast pink" [Huggett and Rowe, 1933], 0·5 c.c. of an 8 p.c. solution per kg., was injected intravenously to prevent coagulation of the blood during manipulation. The common carotid artery being now ligatured and cut, a cannula was tied into its peripheral end and the perfusion started. A small cannula was then tied into the vein to collect the perfusion fluid.

In some experiments the perfusion fluid was Locke's solution of the original composition. In other experiments it was slightly modified to the following composition: NaCl 0·9 p.c., KCl 0·02 p.c., $CaCl_2$ 0·02 c.c., $NaHCO_3$ 0·03 p.c., glucose 0·1 p.c. The significant modification was the reduction of the KCl from 0·042 to 0·02 p.c., since Locke's solution contains sufficient potassium to interfere with some of the pharmacological tests. The above solution, when diluted in the ratio of 1 : 1·4, is almost identical with the original Ringer's solution for the frog. The isolated tissues from frogs and leeches, which were used in some of the tests, were kept in this diluted fluid until the active solutions were actually applied to them, and the perfusion fluid was diluted with water in the ratio 1 : 1·4 for testing. Except in one or two special experiments the perfusion fluid also contained eserine in a concentration of 1/500,000.

Some of the earlier experiments were unsuccessful, owing to failure to realize the necessity for very careful filtration of the perfusion fluid. Filtration through paper is unsuitable for this purpose, and in most of the experiments the perfusion fluid was passed before use through filters made of sintered Jena glass.

The perfusion fluid was contained in a reservoir suspended about 100 cm. above the cat, and connected to the arterial cannula by rubber tubing. In the course of this was interposed a wide glass tube, containing in its upper part an air space through which the fluid passed in drops from a nozzle, the frequency of these giving a rough indication of the rate of perfusion. The fluid then passed through a filter composed of fine linen or glass-wool and thus to the arterial cannula. The arterial cannula

was warmed electrically to about 40° C. by means of resistance wire wound round it. The temperature, controlled by a rheostat, was measured either by a thermometer in a side tube or by a thermocouple. The rate of flow was 0·1–0·3 c.c. per min.

An induction coil was used for stimulating the nerve through platinum electrodes, the primary circuit being interrupted by the automatic hammer. The nerve was moistened continually during the experiment. Stimulation was continued over periods up to ½–1 hour, but it was interrupted during 1–2 min. out of every 3 or 4 min. The distance of the secondary coil was adjusted so that the effect was supramaximal.

The methods used for testing the solutions can be briefly described. For experiments on the cat's blood-pressure chloralose was used as an anæsthetic, the viscera being sometimes removed and eserine injected to sensitize the preparation. The test on eserinized leech muscle was carried out by the method described by Minz [1932] and by Feldberg and Krayer [1933], with minor modifications. In order to economize material the volume of the bath was only 2 c.c. Thin strips of muscle were used, and it was found that the behaviour of symmetrical strips from the same leech was almost identical. The actions of two different solutions can thus be roughly compared by applying one to each strip of muscle. It was found that Locke's solution diluted in the ratio 1 : 1·4 was particularly suitable for use in the bath in these tests. The muscle relaxed rapidly when the acetylcholine was washed out, probably because the solution contained a relatively large amount of potassium, and it was usually possible to detect 0·002γ of acetylcholine, which corresponds to a concentration of 2γ per litre (10^{-9}). Solutions were also tested on the frog's heart isolated by Straub's method, on the frog's rectus abdominis [Chang and Gaddum, 1933] and on the rabbit's auricle [Clark, 1921]. All three preparations were treated with eserine. The last two were suspended in baths of 2 and 3 c.c. respectively.

Results with different tests.

Five different pharmacological methods have been used to test the perfusion fluids for activity. In every case fluid collected during stimulation has been found to have an action indistinguishable from that of acetylcholine, while fluid collected either before or after stimulation was quite inactive. The results obtained in these different tests will be considered separately. The choice of methods is limited by the fact that the activity of the solutions is too small to be detected by any but the most

sensitive tests. The rabbit's intestine is unsuitable as a test object, because of the presence in the perfusion fluid of sufficient eserine to interfere seriously with the behaviour of the intestinal muscle.

Arterial blood-pressure of a cat.

Fig. 1 shows a record of the arterial pressure of a cat (cf. also Fig. 7). 1 c.c. of perfusion fluid collected before stimulation of the nerve was

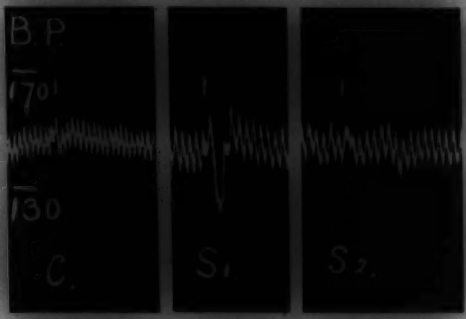

Fig. 1. Cat B.P. S_1 and S_2, fluid collected from the ganglion during stimulation; S_1, before and S_2 after atropine. C, control fluid; no stimulation.

Fig. 2. Cat B.P. Fluid collected from the ganglion during stimulation. Treated A with dilute acid; B with dilute alkali.

injected at C and had no action. On the other hand, 1 c.c. of fluid collected during stimulation caused a fall of blood-pressure (S_1). This effect did not occur when the same injection was repeated after the intravenous injection of 0·1 mg. of atropine (S_2).

Fig. 2 shows that the active substance, producing this fall of blood-pressure in cats, was unstable in alkali. In this experiment the special

saline solution, of which the formula is given above, was used. Fluid collected during stimulation was divided into two portions of 0·9 c.c. each. To one 0·1 c.c. of $N/1$ NaOH was added and to the other 0·1 c.c. of $N/10$ HCl. Both solutions were kept for nearly 40 min. and then neutralized, by the addition of 0·1 c.c. of $N/1$ HCl or $N/10$ NaOH, and injected intravenously into a cat. The portion kept in acid solution was still fully active (*A*) and the portion kept alkaline was inactive (*B*). The effect on the cat's blood-pressure is a particularly suitable reaction for experiments of this kind, because it is unaffected by small changes in the acidity and tonicity of the solution.

Leech muscle.

Eserinized leech muscle provides the most sensitive of the tests we have used for acetylcholine. The effect of fluid collected during stimulation is shown in Fig. 3*A*, and Fig. 6*A*. In these figures the small abrupt rise of the record which precedes the contraction of the muscle is not a specific effect, but is due to the changing of the fluid in the bath. The tracing labelled *D* in Fig. 6 shows that fluid collected several minutes after stimulation was inactive. The small rise in the record which this fluid produced is indistinguishable from that produced at *R* by a simple change of the solution in the bath.

Fig. 3. Above: leech muscle treated with eserine. Below: untreated leech muscle. Effect of fluid collected from the ganglion during stimulation; diluted 1 : 14.

Fig. 3 shows that leech muscle which had not been sensitized to acetylcholine by means of eserine was also insensitive to the active solutions obtained from the ganglion. This experiment was complicated by the fact that the active solutions themselves contained sufficient eserine ultimately to sensitize the muscle, and success depended on the fact that the sensitization was a slow process. This made it possible to show the contrast between the reaction of a piece of muscle which had been previously sensitized and that of one which had not. In the latter case solutions of acetylcholine and active solutions obtained from the ganglion both produced a contraction which was feeble, and which did

not begin till several minutes after the solution had been applied to the muscle. In the experiment shown in Fig. 3 the right and left halves of the anterior part of the muscle from the back of a leech were used for comparison. The left half had been suspended for half an hour in a solution containing eserine (1/100,000); the right half had been kept in a solution free from eserine. The same sample of perfusion fluid, collected during stimulation of the nerve, was tested on both pieces of muscle in a final dilution of 1/14. This degree of dilution was sufficient to make the solution inactive on the normal muscle, though it produced a definite effect on the eserinized muscle. Later, the right half of the muscle was also eserinized, and was then found to be even more active than the left half, both to acetylcholine and to fluid collected during stimulation. The contrast shown in Fig. 3 was therefore due to eserine, and not to asymmetry of the leech. In another experiment it was shown, by a method similar to that described above, that the substance acting on the leech was unstable in alkali.

Frog's rectus abdominis.

The upper part of Fig. 7 shows at *S* the effect on the eserinized frog's rectus abdominis of fluid collected from the perfused ganglion. Fluid collected before stimulation was absolutely ineffective (*C*), while fluid collected during stimulation produced a contraction similar to that due to acetylcholine.

Another portion of the same sample was also tested on the corresponding piece of rectus muscle, taken from the other side of the same frog, but not treated with eserine. This muscle was less sensitive than the eserinized one, both to acetylcholine and to the active perfusion fluid. The latter, however, produced a definite effect, and the estimate of its activity in terms of acetylcholine agreed roughly with the estimate obtained in the experiments with the eserinized rectus, the result of which is shown in Fig. 7.

Frog's heart. (Straub's method.)

Fig. 4 and the upper part of Fig. 6 show the effect on the frog's heart of fluid collected from the perfused ganglion. The effect is indistinguishable from that of acetylcholine and is abolished by atropine (Fig. 4). Fluid collected during stimulation was active and fluid collected in the absence of stimulation was not (Fig. 6 *D*).

Rabbit's auricle.

Fig. 5 shows the effect on the rabbit's auricle. At *S* fluid (0·5 c.c.) collected during stimulation was added to the bath and at *C* fluid (0·5 c.c.) collected without stimulation. The latter had no action. The effect of the active solution was abolished by atropine.

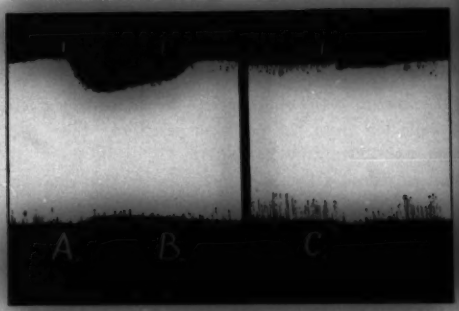

Fig. 4.

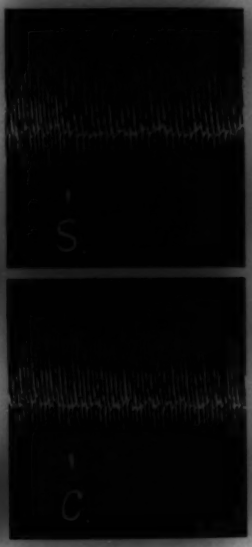

Fig. 5.

Fig. 4. Frog's heart (Straub's method). *A*, fluid collected from the ganglion during stimulation: *B*, 1γ atropine: *C*, same as *A* after atropine.

Fig. 5. Rabbit's auricle. Eserine concentration of the bath 2×10^{-6}. *S*, fluid collected from the ganglion during stimulation: *C*, control fluid; no stimulation.

The identification of the active substance as acetylcholine.

The evidence which has been presented shows that the perfused solution acquired during stimulation various different pharmacological properties similar to those of choline, or a choline ester. The resemblance extends to the fact that the effects on the cat's blood-pressure, the frog's heart and the rabbit's auricle were abolished by atropine. In the case of

several tests there is evidence that the effect was not due to choline itself. Thus the substances acting on the cat's blood-pressure and the leech were unstable in alkali, and the effects of the substances acting on the leech and the frog's rectus were increased by eserine. These facts suggest that one or more choline esters were present in the solution. Since acetylcholine is the only choline ester which has hitherto been isolated from animal tissues, we have made some experiments to test the theory that all these effects were due to the presence of acetylcholine. If an unknown substance, in quantity too small for chemical recognition, shows the pharmacological properties of a choline ester, it can be more closely identified by making simultaneous quantitative comparisons of its activity with those of different pure esters, by different pharmacological methods [Chang and Gaddum, 1933]. If in these different quantitative comparisons the activity of the unknown shows a constant relation to that of a pure ester, the latter is very probably identical with the unknown active principle responsible for all the effects.

Comparisons of this kind are shown in Figs. 6 and 7. In these figures the concentrations given are not the actual final concentrations in contact with the tissue, but the concentrations of standard solutions which, in the process of testing, were diluted to the same extent as the perfused fluid. Thus Fig. 6 shows that the effect of the active fluid, both on the frog's heart and the leech muscle, was greater than that of a solution containing 15γ of acetylcholine per litre and less than that of a solution containing 30γ per litre. In the test on the leech all these solutions were diluted three times so that the actual concentrations of acetylcholine were 5 and 10γ per litre. In the test on the frog's heart they were diluted to an unknown extent by adding 0·4 c.c. in each case to the same, but not accurately known, volume of solution in the cannula.

Fig. 7, which is taken from another experiment, shows that in this case the effect of the active solution, both on the eserinized frog's rectus and on the cat's blood-pressure, was more than that of 30γ of acetylcholine per litre and almost equal to, but in both cases slightly less than that of 50γ per litre. In the experiment on the rectus the solutions were diluted 1 : 1·4 times. The effects on the blood-pressure were obtained by injecting 1 c.c. of each solution undiluted.

In two other experiments similar comparisons were made between the effects on the cat's blood-pressure and those on the frog's heart and the rabbit's auricle respectively. In both cases the results agreed quantitatively with one another, in much the same way as they did in the experiments shown in Figs. 6 and 7. It would not, of course, be possible

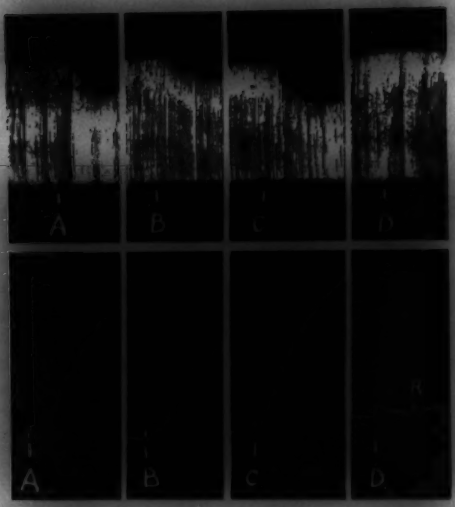

Fig. 6. Above: frog's heart (Straub's method). Below: leech muscle treated with eserine. *A*, fluid collected from the ganglion during stimulation: *D*, control fluid: *B*, *C*, acetylcholine (15 and 30γ per litre respectively).

Fig. 7. Above: Frog rectus abdominis treated with eserine. Below: Cat B.P. *S*, fluid collected from the ganglion during stimulation: *C*, control fluid; no stimulation. A.C. acetylcholine.

in experiments of this kind to detect very small quantitative differences between the results of different tests. If larger quantities of active solution had been easily available more accurate comparisons could have been made, but the results were probably accurate enough for the purpose in view. If the tests on the normal and on the eserinized rectus are considered as two tests, these experiments show that six different pharmacological tests agreed in the quantitative estimate which they gave of the potency of the active solution in terms of that of acetylcholine. Three of these tests are concerned with the muscarine-like actions of acetylcholine.

Since, it is well known, the relative potencies of different choline esters may vary widely when different methods of testing are used, such results provide strong presumptive evidence for the view that the effects seen in all six tests were, at least, mainly due to acetylcholine, and not to other choline esters.

The rate of liberation of acetylcholine under different conditions.

When the perfusion fluid contained no eserine, no activity was detected in any of the tests even when the nerve was stimulated. The addition of eserine to the perfusion fluid did not by itself cause the appearance of activity, as it does in the case of the intestine [Feldberg and Kwiatkowski, 1933] or stomach [Dale and Feldberg, 1933], and no activity was detected in fluid collected without stimulation of the cervical sympathetic, except occasionally in the first sample collected after the perfusion was started. In some cases the sensitivity of the tests on leeches was such that 1·5γ of acetylcholine per litre would have been detected. Such quantities were not present in the absence of stimulation.

The concentration present in the fluid collected during stimulation varied between 20 and 85γ per litre. Since the rate of flow was 0·1–0·3 c.c. per min., a normal rate for the passage into the perfusion fluid of acetylcholine liberated during stimulation would be of the order of 0·01γ per min.

The effects of injections into the perfusion fluid.

The perfused ganglion forms a convenient preparation for the study of the effects of drugs on a ganglion. Under the conditions of our experiments the injection of acetylcholine into the fluid flowing to the ganglion caused a contraction of the nictitating membrane, but the concentration

necessary to produce this effect was usually slightly higher than the concentration present in the fluid collected during stimulation. In some experiments a solution containing 100γ of acetylcholine per litre produced a definite response, but in others it was necessary to use concentrations 100 times as large as this. We have repeatedly tried to confirm Kibjakow's observation that the reinjection of fluid collected during stimulation caused a contraction of the nictitating membrane. If the ganglion was perfused with plain Locke's solution, as in Kibjakow's experiments, we always obtained negative results. In two of the experiments in which the ganglion was perfused with Locke's solution containing eserine, reinjection of the stimulation effluent caused well-marked contractions of the membrane, but in numerous others no perceptible effect was obtained. This was to be expected, since the concentration of acetylcholine, in the venous fluid detected by the other tests, was usually insufficient to have a detectable action on the ganglion, even when eserine was present.

Discussion.

Evidence has been presented that stimulation of the cervical sympathetic nerve causes the liberation of acetylcholine under the conditions of the experiment. The superior cervical ganglion was not the only tissue perfused, but it is the only tissue from which the acetylcholine is likely to have come. The adjacent vagus ganglion is purely a sensory ganglion, and there is no reason to suppose that it would be affected by stimulation of the cervical sympathetic nerve. The other tissue in the neighbourhood, which was also perfused, was insignificant in bulk compared with the two ganglia, and contained no structure having any probable concern with the phenomenon.

Accepting the fact that acetylcholine is liberated in the superior cervical ganglion by impulses reaching it in the preganglionic fibres, we must consider the probability of its function in the transmission of the effect of a nervous impulse at a synapse. Since acetylcholine has a very powerful stimulating action on ganglion cells, the suggestion is obvious that a small quantity liberated at a synapse by the arrival of a preganglionic impulse provides the effective stimulus for the ganglion cell. The observations of Bishop and Heinbecker [1932], Brown [1934] and Eccles [1934] have shown that each preganglionic impulse gives rise to a single impulse in postganglionic fibres, that the delay at the synapse is not more than 2σ, and that the refractory period is very short. If, therefore, the synaptic transmission of each impulse is effected by release of a small charge of acetylcholine, this must occur in close relation to the

ganglion cell; and, since the stimulating effect of excess of acetylcholine on ganglion cells is followed by a depressant action, it must be very rapidly removed after it has transmitted a stimulus. The concentrations found in our experiments in the venous effluent, representing only the acetylcholine which has diffused from the site of its release into the fluid perfusing the blood vessels, cannot be expected, when a small volume is reinjected into the slow arterial stream, to reproduce effects comparable to those of preganglionic stimulation. It would create no difficulty for the theory of impulse transmission by acetylcholine, if the concentration in the venous fluid were too low to have any obvious stimulating action when thus applied. In most of our experiments it had none. There are other cases, in which a humoral transmission is well established, where the dilution found in a venous effluent is too low to reproduce the nervous effect with direct application. On a few occasions, however, arterial reinjection of a small volume of the actual venous fluid has produced definite stimulation of the ganglion cells, as shown by contraction of the nictitating membrane; and we have regularly obtained powerful stimulation by similarly injecting, in 0·1 or 0·2 c.c., a dose of acetylcholine comparable to the quantity appearing in 1 or 2 c.c. of the fluid during a few minutes of stimulation. It is safe, therefore, to conclude that the concentration of acetylcholine produced, at the site of its release in the ganglion, by a preganglionic impulse, is such that it must act as a powerful stimulus to the ganglion cells. If its appearance were held to have no functional relation to the discharge from the ganglion cells of the postganglionic impulses, we should have to postulate another form of excitation of the cells, also produced by preganglionic impulses, and also effective in stimulating plain muscle through the postganglionic fibres, but not electrically recognizable as impulses in those fibres. It seems simpler, on the facts yet available, to suppose that the release of acetylcholine is effective in transmitting the known form of nervous activity, in separate impulses, across the synapse.

Certain difficulties must be briefly considered. Kibjakow, whose technique of perfusion we have in essentials adopted, used plain Locke's solution, without eserine. Under such conditions, he obtained, during preganglionic stimulation, a venous effluent which, on arterial reinjection, caused good contractions of the nictitating membrane. Without eserine in the perfusion we never obtained a venous fluid having any stimulating action on ganglion cells, or on any other reactive organ on which we tested it. Under Kibjakow's conditions acetylcholine does not, in our experience, appear in the venous fluid, and, if it did, it would not be

effective on reinjection. Without eserine in the perfusion, arterial injection of acetylcholine is only effective in concentrations far greater than the highest we obtained, with eserine, in the venous fluid. Although we used his method, Kibjakow's results seem to have little relation to ours, and we are frankly unable as yet to explain them.

The demonstrated action of eserine, in protecting acetylcholine on its way from or to the ganglion cells through the blood vessels of the ganglion, raises the question of its influence on the effect of preganglionic stimulation. We found it difficult to predict what its action would be. Its potentiation of effects like those of the heart vagus, where the effect of one impulse on the rhythm is prolonged, and that of a series rendered more intense by summation, owing to the delayed enzymatic destruction of acetylcholine after release, seemed to provide no obvious analogy for its probable effect on transmission through the ganglion. It seemed that, in this case, eserine could only potentiate the effect of a preganglionic impulse, or of a series of such impulses, if it enabled a larger number of ganglion cells to be effectively stimulated. Some experiments, still in progress, by one of us (W. F.) with A. Vartiainen, appear to show a pronounced potentiation by perfusion of a very dilute (1 in 10^9) solution of eserine through the ganglion, the responses to successive short groups of submaximal shocks to the preganglionic nerve being recorded. Earlier experiments, with continued perfusion of stronger solutions of eserine had shown depression of the response as the principal effect. This secondary depressant effect of eserine is the only one detected by Eccles [1934], in his experiments recording the action potentials set up in the ganglion and its postganglionic branches by single and repeated volleys in the preganglionic nerve. His conclusion, from this evidence, that "the liberation of acetylcholine is therefore not responsible for the transmission of impulses through the ganglion," seems to us premature at the present stage. We still believe such transmission to be the most reasonable interpretation of our own results, including the few which we have completed on the action of eserine.

It is tempting to generalize from these conclusions and to suggest that all preganglionic autonomic nerve fibres may be cholinergic [Dale, 1933]. If this is true of parasympathetic as well as sympathetic nerves, the question arises whether the "Vagusstoff" of Loewi may not have been liberated at ganglionic synapses. It is very improbable, however, that it was entirely, or even largely derived from this source. The effects of eserine and atropine on the action of the heart vagus, as well as the analogy from Engelhart's [1931] experiment on the pupil, strongly

support the view that the postganglionic vagus fibres to heart muscle are cholinergic. But it is still possible, and even probable, that the preganglionic vagus fibres are also cholinergic, and, since their ganglionic synapses lie in the heart tissue, their mechanism may also make some contribution to the "Vagusstoff."

Summary.

1. The superior cervical ganglion of a cat was perfused with Locke's solution containing eserine, and pharmacological tests were applied to the fluid emerging from the vein.

2. Stimulation of the cervical sympathetic caused the liberation from the ganglion of a substance which was pharmacologically identified as acetylcholine. The identification depended on quantitative agreement between estimates, obtained by six different methods, of the potency of the active solutions relative to that of acetylcholine.

3. These observations support the theory that the mechanism by which each nerve impulse normally passes the synapse consists in the liberation of a small quantity of acetylcholine.

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612.327.2:547.435–292

THE CHEMICAL TRANSMITTER OF VAGUS EFFECTS TO THE STOMACH.

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(*Received March* 12, 1934.)

Since Loewi [1921] first demonstrated the chemical transmission of the inhibitor effect of vagus impulses on the frog's heart, a similar mechanism has been recognized in the transmission of other peripheral parasympathetic effects. The reluctance of many investigators in this field to identify the transmitter as acetylcholine itself, and still to speak of it as "acetylcholine-like," can be justified by the absence, hitherto, of direct chemical evidence of identification. The resistance of certain parasympathetic effects to atropine, which easily annuls the corresponding effects of acetylcholine, has seemed to others to impose an even stronger objection to such identification; and it must be admitted that an assumption that acetylcholine acts as the general parasympathetic transmitter requires additional suppositions to explain this anomalous difference [cf. Dale, 1929; Dale and Gaddum, 1930]. It should, however, be noted that the action of atropine, if regarded as decisive against acetylcholine itself, is equally so against any choline derivative; so that, if the transmitter on this account is held not to be acetylcholine, it cannot be "acetylcholine-like." Among the parasympathetic effects often cited in connection with this difficulty caused by resistance to atropine, are those of the vagus and the pelvic nerves on the muscular walls of the alimentary canal and the bladder. We decided to attempt to obtain the chemical transmitter effective in these actions, and to test its properties. Hitherto our observations have only been completed for the action of the vagus on the stomach. This action has, for the purpose in view, two disadvantages: the action of the vagus on the stomach muscle is not very highly resistant to atropine, and we have not found it possible to separate the circulation of the muscular wall from that of the mucous membrane, on the glands of which the vagus has a secreto-motor action. On the other hand, the augmentor action of the vagus on the activity of the small intestine, which resists atropine in larger doses, presents additional difficulties for our purpose, in that it has not the same definite relation

to the period of stimulation as has that on the stomach. It seems desirable, therefore, to publish the completed experiments on the gastric vagus, and to leave open to further investigation the less clear-cut effect of this nerve on the small intestine, and the action of the pelvic nerve on the colon.

Methods.

(1) *Natural circulation.* Dogs have been used as subjects of all the main experiments. After preliminary anæsthesia with ether, under which the required cannulæ were inserted, a 1 p.c. solution of chloralose in warm saline was given by slow intravenous infusion to a total of 15–20 c.c. per kg., according to the amount needed to produce a deep anæsthesia, which was maintained by a further infusion, when necessary, in the later stages of a long experiment. The abdominal cavity was opened by a median incision, and with successive ligature of the arteries and veins the whole of the large and small intestines was removed, nearly up to the pylorus. Care was taken in removing the duodenum, which was ligatured a few centimetres below the pylorus, to preserve any branch from the pancreatico-duodenal artery to the pyloric end of the stomach. The main gastric artery to the fundus, and the gastric veins draining into the splenic vein, were carefully preserved in the subsequent removal of the spleen and the remainder of the pancreas. A cannula, filled with saline solution and provided with a clamped rubber tube, was now inserted into the lower end of the portal vein, just above its formation by union of the mesenteric veins. Opening of the clamp on the tube leading from this cannula, accompanied if necessary by compression of the portal vein just below the liver, led to the outflow for collection of venous blood coming entirely from the stomach.

The abdominal wall was now closed, leaving egress for the tube leading from the portal vein. The dog being turned on its right side, a window was made in the left side of the lower chest wall by removing the middle portions of two or three ribs, with the attached intercostal muscles, care being taken to secure complete hæmostasis. The vagi were then dissected off the wall of the œsophagus from just below the roots of the lungs to about 1 cm. above the diaphragm. They were tied and cut above, and their peripheral ends prepared so that they could be laid together across a pair of chlorided silver-wire electrodes, when this was inserted through the window in the chest by a long insulating holder.

The blood of the animal was now rendered incoagulable by an intravenous injection of heparin, or of the dye "chlorazol-fast pink" [see Huggett and Rowe, 1933], which we have found very effective and

suitable for experiments of this kind. Eserine was injected, in a quantity judged to be sufficient to protect acetylcholine or a similarly sensitive choline ester from destruction by the blood esterase, during passage from the site of liberation to the collecting cannula. As will be seen, it is doubtful whether more than partial protection was ever achieved. With the eserine, in a dose of 1–2·5 mg. per kg. of body weight, we gave a small dose—usually a total of 0·5 mg.—of atropine, to prevent a retardation of the heart beat by the eserine sufficient to impair the circulation. The first control sample of portal (gastric) blood was taken about 10 min. after administration of these alkaloids, to allow the inhibitory effect of eserine on cholinesterase to become fully established. Each sample, consisting of 10 c.c. of blood, was taken in measured time into a graduated cylinder, which already contained 0·2 c.c. of 1:1000 eserine, so that the completed sample contained 1 : 50,000 of additional eserine. The activity of the samples was, so far as possible, tested without delay, but, when delay was necessary, the cylinders containing them were immersed in pounded ice. An actual trial showed that a sample containing 1 : 50,000 eserine, and thus cooled to about 0°, could be kept for even an hour without significant loss of activity.

(2) *Artificial perfusion.* As for experiments with natural circulation, the dog was anæsthetized with ether followed by chloralose, and the dissection for removal of the intestines, spleen and pancreas followed a similar course. Great care was taken to tie even the smallest vessels, and the omentum was removed, except for the strip involving the veins draining the stomach into the splenic vein. The hepatic artery was now doubly ligatured near to the liver and cut across, and a ligature laid ready for tying round the portal vein at the same level. The cœlic axis, with the gastric and hepatic arteries, now its only functional branches and supplying only the stomach, was dissected clear from its bed and from the surrounding solar plexus, and made ready for rapid insertion of a perfusion cannula, and for removal from the body immediately thereafter. The chest was then opened widely, under artificial respiration, by splitting the sternum, and the vagi were tied and cut just below the roots of the lungs, and dissected clear from the œsophagus to just above the diaphragm. The œsophagus was then opened, and a wide glass cannula was inserted so as to project into the lumen of the stomach through the cardiac orifice, the œsophagus being tied firmly round the neck of the cannula. The glass cannula was made long enough at the other end to project through the opening of the thermostat chamber, to which the isolated stomach was later removed.

The arterial perfusion cannula was now inserted into the cœliac axis, the portal vein tied near the liver, the cannula in the lower end of the portal vein opened, and perfusion of the stomach with Locke-Ringer solution was begun. The perfusion was carried out by a single-chamber Dale-Schuster pump, immersed in a water bath at about 39° C. A long rubber tube led the warm Locke's solution to the arterial cannula, while the stomach with its attachments was being removed from the body and transferred to the thermostat chamber. This was essentially the chamber described and figured by Bauer, Dale, Poulsson and Richards [1932] for perfusion of the isolated liver. In the present case the wide glass tube, tied into the lower end of the œsophagus, passed out through the gap on one side of the ebonite rim of the chamber, to be fixed by a clamp. Through the same gap passed the insulating holder of the stout silver electrodes, across which the vagi were laid inside the chamber. Through the gap in the opposite side of the rim passed the long beaks of the arterial and venous cannulæ, and a string by which the pylorus was anchored to an external support. The stomach lay in the chamber on a sling of netting, fixed at its cardiac and pyloric ends. The gaps in the rim being filled with putty, the chamber was now closed by the glass lid, previously warmed, and the temperature inside it rapidly rose to about 38° C., which was also that of the solution in the arterial cannula. The air in the chamber was enriched by a stream of oxygen, which bubbled through warm Ringer's solution before entering the air space. The arterial pump was adjusted to give a moderate rate of perfusion, 1 c.c. flowing from the venous cannula in about 2·5–3 sec., or 100 c.c. in 4–5 min.

Only a few minutes elapsed between the removal of the stomach from the body and the closure of the thermostat chamber, and not more than a momentary interruption of the arterial perfusion occurred during the transfer. A change was now made from the plain oxygenated Locke's solution used for this preliminary perfusion to similar Locke's solution containing 1 part of eserine in 300,000–500,000. This caused well-marked spontaneous contractions of the whole stomach, which subsided with continued perfusion. Samples of the control venous fluid could then be taken, and other samples during stimulation of the vagi.

Results.

(1) *Natural circulation.* Section of the vagi cannot be regarded as completely stopping parasympathetic stimuli to the muscle coats of the stomach wall. The ganglionic plexus between the muscle coats, with the

cells of which the preganglionic fibres of the vagus are generally supposed to make connections, keeps the stomach wall in spontaneous activity, apart from impulses from the vagi. That this spontaneous activity is maintained by peripheral liberation of a choline ester is suggested by its intensification by eserine. It is not surprising, therefore, to find that the control samples of portal blood, collected after eserine has been administered, already contain recognizable amounts of a substance resembling acetylcholine in action, when tested on the eserinized leech muscle or on the blood-pressure of the anæsthetized cat. It had already been shown by Feldberg and Rosenfeld [1933] that the portal blood in an animal with intact intestines may show clear evidence of the presence of a substance acting like acetylcholine, after a large dose of eserine has been injected, and without any nerve stimulation. We could not, therefore, in the case of the stomach, obtain a clear-cut difference between "resting" venous blood and "active" venous blood; for there is no resting state of the organ. What we could expect to find was a quantitative difference, in the direction of an increase of the activity due to the chemical transmitter, in the blood taken during effective vagus stimulation, as compared with that of the blood taken before vagus stimulation, or again at a later stage, when the activity due to the stimulation had subsided. Such differences we regularly observed; but we found, in the course of the earlier experiments, that in order to obtain a large difference, so that the activity of the "vagus" blood was 2½ to 4 times that of the "control" blood, it was necessary to stimulate the vagus strongly, so that the stomach wall was thrown into vigorous contractions.

An extract from a protocol will conveniently indicate the procedure and the nature of the results of an experiment.

Exp. 1. Dog. 13 kg.

Experiment begun 10 a.m. Anæsthesia and dissection as under "Methods."

1 p.m. A cat was anæsthetized with ether followed by chloralose and prepared for testing samples on blood-pressure. Two symmetrical strips of the body wall of a leech suspended in aerated Ringer solution and sensitized by addition of 1 : 200,000 eserine. Eserine in a concentration of 1 : 50,000 was added to all blood samples as collected, except when otherwise stated.

1.50 p.m. Blood of dog made incoagulable with "chlorazol-fast pink." Samples of portal blood, tested in 1 in 4 dilution had a slight, non-specific action on the leech preparations with a first, but not with a renewed application.

2.8 p.m. 0·5 mg. atropine and 30 mg. eserine intravenously.

2.20 p.m. Control sample I of portal blood. Tested on leech. In 1 : 4 dilution it produces a rapid contraction (Fig. 1, *A*), corresponding to that earlier produced by A.C.[1] $1 : 200 \times 10^6$. The concentration in the undiluted blood is, therefore, about $1 : 50 \times 10^6$.

[1] The symbol A.C. is here used for brevity to represent acetylcholine.

2 c.c. undiluted blood, injected intravenously into cat, produce just perceptible fall of arterial pressure. Effect too small for accurate measurement.

2.43 p.m. Control sample II. Tested on leech in 1 : 10 dilution (probably about $1 : 500 \times 10^6$ A.C.) causes very small slow contraction (Fig. 1, *B*).

2.50–2.52 p.m. Stimulate vagi in thorax, with secondary coil at 6 cm. Powerful and continuous contractions of stomach wall. Blood samples III and IV collected during, and sample V after end of stimulation. Samples III and V were collected with the normal addition of 1 : 50,000 eserine, and placed in ice. Sample IV was collected without additional eserine and kept at room temperature.

2.52 p.m. Sample III tested for action on cat's blood-pressure in comparison with a standard solution of A.C., $1 : 10^9$. Repeated doses of 2 c.c. of the undiluted blood produced falls of arterial pressure of the same depth as those produced by 0·1 γ of A.C. After 0·1 mg.

Fig. 1. Leech muscle preparation in eserine 1 : 200,000. See protocol of Exp. 1.

of atropine a further 2 c.c. of blood caused only a slight rise of blood-pressure. The action of the blood would be accounted for by the presence in it of A.C. to the extent of $1 : 20 \times 10^6$.

2.53 p.m. Sample III tested on the leech in 1 : 10 dilution, causing a contraction (Fig. 1, *C*) similar to that (Fig. 1, *A*) caused by sample I in 1 : 4 dilution. Again the activity of sample III is estimated at $1 : 20 \times 10^6$ A.C., or $2\frac{1}{2}$ times that of sample I.

3.35 p.m. Sample IV, kept at room temperature without additional eserine for 43 min., tested on leech in 1 : 10 dilution. No effect (Fig. 1, *D*).

3.51 p.m. Sample V, kept at 0° C. with added eserine for nearly 1 hour, tested similarly in 1 : 10 dilution, producing a contraction (Fig. 1, *E*), only slightly less rapid than that in Fig. 1, *C*. Sample V, collected just after stimulation, may have been initially a little less active than sample III; or the leech preparation may have been declining in sensitiveness. Fig. 1, *D* and *E* show, in any case, that the substance appearing in the gastric venous blood on vagus stimulation is relatively stable at 0° C. in the presence of additional eserine, and rapidly disappears from the blood at room temperature and without added eserine.

3.53 p.m. Another control sample, VI, tested on the leech in dilution 1 : 10, had no

effect (Fig. 1, *F*). In 1 : 4 dilution it produced a contraction comparable to that of Fig. 1, *B*. Probably its activity, therefore, was not much higher than that of A.C. $1 : 100 \times 10^6$.

4.2–4.3½ p.m. Renewed vagus stimulation, during which sample VII of portal blood was collected. Tested on the leech, in comparison with dilutions of acetylcholine, it produced a contraction corresponding to an original concentration of A.C. $1 : 25 \times 10^6$.

Experiment terminated.

It will be seen that such an experiment demonstrates that:

(1) There appears in the portal (gastric) venous blood, after injection of a large dose of eserine, a substance not previously present, which has on the eserinized leech muscle a stimulating action comparable to that of acetylcholine in high dilution.

(2) On stimulating the vagi this activity of the portal blood shows a 2½ fold increase, and the blood now shows a depressor action on the cat's blood-pressure, matched by acetylcholine in a concentration identical with that matching the stimulant action on the leech. This action on the blood-pressure is completely abolished by a small dose of atropine.

(3) An hour after the stimulation, the specific activity of the portal blood is lower than before stimulation. Renewed stimulation of the vagi raises it again to a level close to that attained in the first stimulation.

(4) The substance appearing in the blood on vagus stimulation disappears rapidly from the shed blood at room temperature, but it persists for one hour, with little or no loss, if the blood is treated with eserine and kept at 0° C.

In another experiment a sample of the gastric venous blood, collected during vagus stimulation under conditions similar to the above, was tested in the same (1 : 10) dilution on two symmetrical preparations from the same leech, of which one had been given preliminary treatment for half an hour with 1 : 100,000 eserine, while the other was suspended in plain Ringer's solution. The results are shown in Fig. 2, *A* and *B*. It will be seen that the characteristic stimulant action shown in record *A* is entirely absent in *B*. Although the blood contained eserine in sufficient quantity, even after dilution, eventually to sensitize the leech, the process is a slow one.

Fig. 2. Symmetrical strips of leech, *A* in eserine 1 : 100,000, *B* in plain Ringer. Application to both of "vagus" blood samples diluted 1 : 10.

The substance appearing in the blood from the stomach during vagus stimulation is, accordingly, one which is destroyed

by the blood, but protected by addition of eserine, and one of which the stimulating action on leech muscle is very much enhanced by preliminary treatment of the muscle with eserine.

In another experiment, otherwise similar to that described above in detail, a smaller dose of eserine (1 mg. per kg.) was injected into the dog, and the control sample of gastric venous blood showed a lower activity, corresponding, in the test on the eserine-treated leech approximately to that of A.C. $1 : 200 \times 10^6$. The activity of the blood collected during vagus stimulation showed in this case a fourfold increase, corresponding to A.C. $1 : 50 \times 10^6$. The same samples, and another collected during a subsequent period of vagus stimulation, were tested more thoroughly on

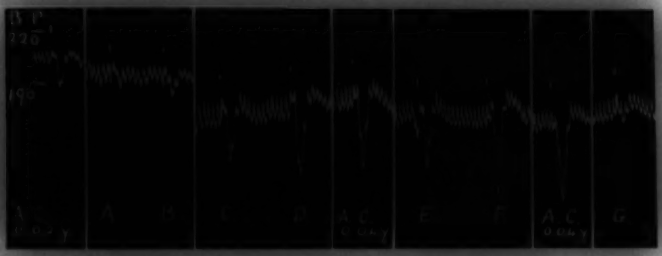

Fig. 3. Cat under chloralose. Carotid blood-pressure. At *A* and *B* 1 and 2 c.c. of control blood, at *C* and *E* 1 c.c., at *D*, *F* and *G* 2 c.c. of two "vagus" samples. Before *G* 0·1 mg. atropine. For details see text.

the blood-pressure of a cat under chloralose, an attempt being made to balance their activities against those of known doses of acetylcholine. Fig. 3 shows the result. Injections at *A* and *B*, of 1 and 2 c.c. respectively of the control portal blood, produce effects too small to be balanced accurately against acetylcholine. It can safely be estimated, however, that the effect of 2 c.c. at *B* is less than that of the preliminary dose of $0{\cdot}02\gamma$ A.C.; so that the blood is definitely weaker than A.C. $1 : 100 \times 10^6$, and probably not stronger than A.C. $1 : 200 \times 10^6$, which matched it in the test on the leech. The effect, at *C*, of 1 c.c. of blood collected during the first vagus stimulation, and at *D* of 2 c.c. of the same blood, can be compared with that of $0{\cdot}04\gamma$ A.C. which follows them immediately; and it can be seen that 1 c.c. causes a smaller, and 2 c.c. a somewhat larger effect than the control dose of A.C. This sample, accordingly, is somewhat stronger than A.C. $1 : 50 \times 10^6$. At *E* and *F* injections of 1 and 2 c.c.

were made of a sample collected during a second period of vagus stimulation. In this case the effect of 2 c.c. is equal to or very slightly less than that of 0·04γ A.C. We may tabulate the results of these estimates as follows.

Blood sample	Equivalent activity in concentration of A.C.
Control	$< 1 : 100 \times 10^6$; not $> 1 : 200 \times 10^6$
1st vagus sample	$\leqq 1 : 50 \times 10^6$
2nd ,,	$\leqq 1 : 50 \times 10^6$

In both vagus samples, therefore, the activity on the cat's blood-pressure, as on the leech muscle, was approximately 4 times that of the control sample, and matched the same concentration of A.C. as in the leech experiment.

Finally the cat received an intravenous injection of 0·1 mg. of atropine, and, at *G* in Fig. 3, a further injection of 2 c.c. of the second sample of vagus blood, similar to that given at *F*, was now without significant action.

(2) *Artificial perfusion.* The general results of these experiments were very similar to those with natural circulation. The method had certain advantages, however, which enabled us to make some additional tests. The rate of the perfusion could be very closely controlled by adjustment of the stroke of the pump. With natural circulation we were, of course, obliged to take the venous blood at the rate at which it entered the portal vein. We did not, in fact, observe any important change in the rate during vagus stimulation; but the rate of outflow, both in control and vagus periods, was only controlled, and then not very exactly, during the time of collection of a 10 c.c. sample in each case. With artificial perfusion the whole effluent could be collected for any required period. We regularly, indeed, took a sample of 100 c.c. of control fluid, recording accurately its time of collection, and again a sample of 100 c.c. during vagus stimulation. The outflow under the latter conditions showed an obvious retardation during the initial strong contractions caused by the onset of vagus stimulation. This might be compensated naturally by accelerated flow later in the period; if not, the stroke of the pump was slightly increased, so as to make the time for collection of the full sample of 100 c.c. practically equal to that for the control sample.

As to the direct comparisons of the activities of the control and stimulation fluids, as collected, it need only be said that they showed similar ratios to those obtained for the corresponding samples of blood with natural circulation, varying from about 1 : 2·5 to 1 : 4 in different experiments. The estimates in terms of acetylcholine, also, corresponded

again for the "muscarine" action on the cat's blood-pressure, and the "nicotine" action on the leech. The fluid being Locke's solution instead of blood, it was further possible to use the isolated frog's heart for direct comparisons, the fluids being diluted with water to 1·4 times their volume, and tested on a heart previously beating on the unperfused eserinized Locke's solution diluted to the same extent. Fig. 4 shows the effects on such a heart, at *A* of control fluid collected before stimulation, at *B* of fluid collected during it, at *C* of fluid collected, 8 min. after it ended, at *D* of fluid collected during a second period of vagus stimulation,

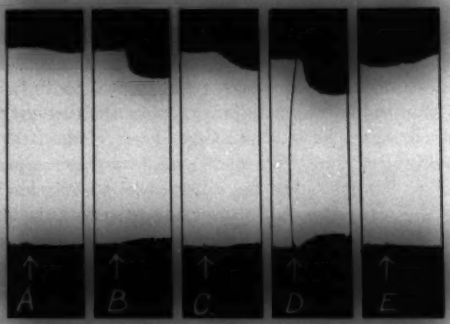

Fig. 4. Isolated frog's heart (Straub). Ringer contains 1 : 700,000 eserine. Tested with perfusion effluents collected: *A*, before stimulation; *B*, during first vagus period; *C*, 8 min. later; *D*, during second vagus period; *E*, same as *D*, after atropine.

and at *E* of the same fluid as at *D*, after treating the heart with a trace of atropine.

The main advantage of the experiments with artificial perfusion, however, was the possibility of collecting a comparatively large volume of fluid, containing the transmitter of the vagus effect in a relatively clean solution. The saline effluent containing it could be taken to dryness by evaporation under diminished pressure, the inorganic salts eliminated, except for traces, by extracting the carefully dried residue with absolute alcohol, and, after removal of the alcohol by renewed evaporation, the transmitter could be obtained in solution at any desired concentration, and in a quantity sufficient to enable its activity to be determined within fairly narrow limits, in terms of acetylcholine, by a series of different physiological reactions. For this purpose the venous effluents were

collected in graduated vessels already containing the small volume of $N/10$ HCl required to bring the reaction of the total quantity collected to about pH 4, at which acetylcholine is very stable in watery solution. Sufficient eserine being present to suppress the action of any cholinesterase, such fluids could be evaporated under diminished pressure, the process being at intervals interrupted to adjust the reaction, so that the acidity did not seriously exceed pH 4.

In this way samples of 100–200 c.c. of control and stimulation fluids were treated, so that the activity was contained in any desired fraction of the original volume of Locke's solution, without excess of salt. The eserine present in the original fluid was not eliminated; but even assuming that it was concentrated without destruction, an original concentration of $1 : 5 \times 10^5$, which was adequate to protect the transmitter under the conditions described, even if concentrated tenfold to $1 : 5 \times 10^4$, would still give a practicable concentration for presensitizing preparations of leech muscle, or of the skeletal muscle or heart of the frog, and would have no disturbing effect on tests made with intravenous injections into a cat under chloralose. Tests on the control and stimulation fluids, concentrated in this way, showed that they had preserved the original ratio of their activities, with but little absolute loss. Attention was accordingly directed, in two experiments, to a more careful determination of the activity of the fluid collected during stimulation, in a series of different physiological reactions, in terms of acetylcholine. The two experiments gave quite similar results, and it will suffice to record them in greater detail and illustrate them in the case of one.

In the particular experiment from the records of which Fig. 5 was constructed, the unconcentrated control and stimulation fluids gave the effects on the frog's heart shown in Fig. 4. A relatively low concentration (1 : 500,000) of eserine had been used for the perfusion, and even the stimulation fluid had an activity of only about A.C. $1 : 80 \times 10^6$. After evaporation to dryness, however, and removal of salts, a solution having one-third of the original volume, and therefore about three times the original activity, was found suitable for the test on a cat's arterial pressure. For the tests on the eserinized frog's heart and skeletal (rectus abd.) muscle the concentrate was further diluted with 40 p.c. of its volume of distilled water. For the test on the sensitive leech preparation it required further dilution with 5 times its volume of the appropriate Ringer's solution—*i.e.* to less than half the original strength. The strength of the threefold concentrate was first determined on the cat's blood-pressure. In Fig. 5 *a*, *A* and *C* show the effects of injections of 1 c.c. each of

A.C. $1 : 40 \times 10^6$ and $1 : 20 \times 10^6$ respectively, and *B* that of 1 c.c. of the concentrate. It will be seen that the action of the latter is "bracketed" by those of the two doses of acetylcholine, and, so far as can be estimated, lies about midway between them. For the other tests these two stock dilutions of acetylcholine were diluted in exact parallel with the concentrate, so that the ratios between the doses giving the different effects at *A*, *B* and *C* remained constant, at the different dilutions above indicated. It will be seen that the effect at *B* is similarly bracketed by those at *A* and *C* in every test, and that the activity of the concentrate is approximately midway between those of the two dilutions of acetylcholine. We may conclude, accordingly, that the activity of the transmitter, released by stimulation, is constant in relation to that of acetylcholine in all four tests, and that the activity of the concentrated effluent, before further dilution, was in each case approximately equivalent to that of A.C. $1 : 30 \times 10^6$.

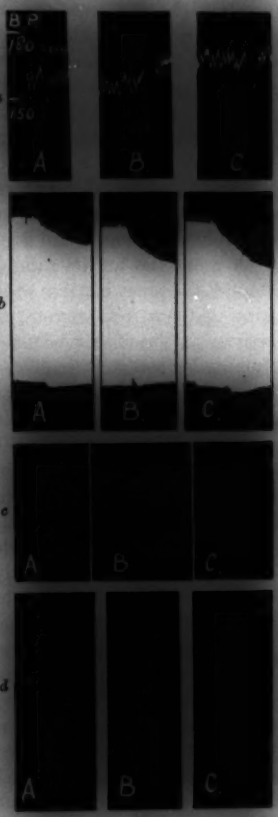

Fig. 5. Tests of concentrated vagus effluent on: *a*, blood-pressure of cat under chloralose; *b*, frog's heart (Straub); *c*, frog's rectus abdominis; *d*, leech muscle. In each case *B* shows the effect of the perfusion concentrate, given in constant ratio to two strengths of A.C., at *A* and *C*, that at *C* being double that at *A*. See text.

In two experiments the stability of the transmitter was tested by making the solution strongly alkaline and acid respectively; with the result that the activity disappeared rapidly from the alkaline solution at room temperature, but remained practically unchanged in the acid solution under the same conditions.

One further experiment may be mentioned, in which the concentrated stimulation fluid, when injected into the aorta through the stump of the cœliac artery in an eviscerated cat, produced an output of

adrenaline from the suprarenal gland, exactly comparable to that produced by the corresponding dose of acetylcholine [cf. Feldberg and Minz, 1931].

Discussion.

The active substance which is found already in the venous effluent from the stomach under eserine, in the absence of vagus stimulation, shows such a pronounced increase—as much as fourfold—during vagus stimulation, that there is no reason for doubting its function as the transmitter of the effects of vagus impulses to the active structures of the stomach wall. In all the properties which a variety of tests revealed, this substance was found to be indistinguishable from acetylcholine. Such properties are its rapid destruction by blood in the absence of eserine; the great increase of its activity on different tissues caused by treating them with eserine; the constant quantitative relation of its activity to that of acetylcholine in different tests, some depending on the "muscarine action" others on the "nicotine action" of that substance; the annulment of its "muscarine" actions by small doses of atropine; and its instability to alkali, even in the presence of a protective concentration of eserine. There is no reason to doubt that it is acetylcholine, though our hope that, by artificial perfusion, we might be able to obtain the transmitter in sufficient concentration to justify an attempt at chemical isolation, has not been realized.

The point of liberation of this acetylcholine by impulses in the vagus is less certain. We have already mentioned the possibility that it might come from the mucous membrane, rather than the muscle coats. We regard this possibility as discounted by the fact that we only observed a large increase in the output when stimulation of the vagus threw the muscular wall into vigorous contraction. The fact that, as McSwiney [1933] has shown, the augmentor effect of vagus impulses on the stomach musculature is enhanced by eserine, was already in favour of the transmission of such effects by a choline ester. Even if we assume, however, that vagus impulses concerned with increase of muscular activity are responsible for liberation of at least an important part of the acetylcholine detected, we must still, in the light of the latest evidence [cf. Feldberg and Gaddum, 1934], consider whether the release occurs in transmitting preganglionic impulses to the ganglionic plexus, or in transmitting the effect of postganglionic impulses to the plain muscle fibres. It is possible that both may be concerned. The considerations suggested by Feldberg and Gaddum (*loc. cit.*) as to the origin of the acetylcholine released in the heart by vagus impulses, apply probably with greater

force to the case of the stomach wall, with its rich ganglionic plexus. It must be admitted, however, that the probabilities with regard to several of the points involved are derived from what may be misleading analogies. Thus, it is probable that parasympathetic preganglionic fibres are cholinergic, as those of the true sympathetic system are now known to be on direct evidence; and it is probable that the action of acetylcholine on parasympathetic ganglion cells is of the "nicotine" type, resistant to atropine, as it was long ago shown to be on true sympathetic ganglion cells [Dale, 1914]. Neither of these probabilities, however, has been brought to the test of direct experiment, and the difficulties of doing so will not be readily surmounted. We must be satisfied meanwhile to regard it as probable that the transmission of preganglionic vagus impulses to the cells of the ganglionic plexus in the stomach, and that of post-ganglionic impulses to the plain muscle cells, are both dependent on release of acetylcholine, and that the sensitiveness of this vagus effect to atropine is due to blockage of the "muscarine" effect on the plain muscle, rather than of the transmission at the ganglionic synapses.

It should be emphasized that we have not observed any kind of physiological activity in the venous effluent from the stomach, increasing with vagus stimulation, except that due to a substance indistinguishable from acetylcholine; and, further, that this increase of acetylcholine in the venous fluid must correspond to such an increase at the actual site of liberation as would account for all the augmentor effects of the vagus on the activity of the stomach, with which we have been concerned. There is no reason for differentiating these effects from those of the vagus on the heart, with regard to the chemical mechanism of their transmission. If acetylcholine is accepted as the transmitter in both these cases, it may further be regarded as very improbable that the mechanism should suddenly change at the pylorus, and that the augmentor effects of the vagus on the activity of the small intestine should be transmitted by a radically different method. The evidence now obtained as to the method of transmission in the stomach, seems to us, therefore, to weaken further the significance which some have accorded to the relatively high resistance to atropine of the effects of the vagus on the small, and of the pelvic nerve on the large intestine.

Summary.

The venous blood, or perfusion fluid, from the stomach wall contains acetylcholine in recognizable concentration, when eserine has been injected or added to the perfusion.

Stimulation of the thoracic vagi, adequate to cause contractions of the stomach wall, causes a manifold increase in this acetylcholine.

Acetylcholine acts as the chemical transmitter of vagus effects to the stomach.

It is a pleasure to acknowledge help given in some experiments by Dr H. W. Dudley and Dr A. Vartiainen.

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THE ASSAY OF THE OVULATION-PRODUCING SUBSTANCE.

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(*Received March* 6, 1934.)

The induction of ovulation in the unmated œstrous rabbit by the administration of anterior pituitary extracts was first demonstrated by Bellerby [1929]. Shortly afterwards Friedman [1929] obtained the same effect by the injection of extract of urine of pregnancy. Since these original observations, the reaction has become a recognized test for one particular type of gonadotropic activity, *i.e.* causation of ovulation in ripe follicles.

In practical use, assay on œstrous rabbits of the ovulation-producing capacity of extracts has many conveniences, notably the fewness of the variables when compared with other systems of assay and the fact that the animals can be used more than once. Up to the present, however, no attempt seems to have been made to achieve an accuracy comparable with that commonly given by the biological assay of œstrin. No attempt was made by Bellerby, by Friedman in his first experiments, or by Hill and Parkes [1930] to consider individual variation in response or other variables. Much of the work done on the rabbit ovulation test has been in connection with pregnancy diagnosis [Friedman and Lapham, 1931; Wilson and Corner, 1931], which depends largely on qualitative examination of the urine and does not involve accurate quantitative examination.

In a later paper, Friedman [1932] stated that if post-partum œstrous animals only were used, there was little individual variation in response; he considered the least amount causing ovulation in a series of rabbits receiving various doses as the minimum effective dose (M.E.D.). White and Leonard [1933] arrived at what they called the "minimum

ovulating dose" (M.O.D.) by a somewhat similar technique, but made no claim to great accuracy. In our experience, post-partum rabbits are no more reliable than ordinary non-pregnant animals isolated for three weeks[1], and in any series injected with graduated amounts, there is overlapping between the positives and the negatives, unless there are such large intervals between the various doses that the assay is useless. In other words, no exact meaning can be attached to the term "minimum effective dose." This point has been emphasized again and again in the assay of œstrin and other substances [see Coward and Burn, 1927].

The following paper gives an account of a detailed investigation into various factors, notably individual variation in response, influencing the reaction of œstrous rabbits to gonad-stimulating preparations. The well-known method of considering the percentage response of an adequate group of animals (about twenty) was used. The results make it evident that the test can be put on a quantitative basis and can be made to give reasonable accuracy.

I. Possible variables in the rabbit ovulation test.

A single intravenous dose is now generally used in carrying out the rabbit ovulation test, and since this technique is both convenient and highly effective, no good purpose can be served by considering any other. Site of administration, number of doses and interval between doses are thus settled immediately. Similarly the medium is automatically determined; aqueous solutions or fine emulsions are essential, and oil injections which involve uncertainty as regards completeness of absorption are out of the question. Within limits, the volume of the injection, which is very small in relation to the blood volume, cannot be important. Further, there is no problem as to what constitutes a positive reaction, either ovulation occurs or it does not, and in scarcely one case in a hundred is there any trouble in diagnosing ovulation by ordinary macroscopic examination of the ovaries. The time of making the examination also offers no difficulty; injection is made on one day and a positive result will be visible on the next day as ruptured follicles, or later as corpora lutea. It is thus apparent that out of eleven possible variables in the assay of œstrin listed by Marrian and Parkes [1929] six are immediately eliminated in the rabbit ovulation test, one of the remaining five (time after ovariectomy) is not relevant and another (age of the animal) is not

[1] Friedman's [1932] statement that post-partum rabbits "invariably accept the male and invariably ovulate after coitus" cannot be accepted. Invariability is not a biological phenomenon.

likely to be a serious factor provided that ripe follicles are present. There remain only three possible variables to be dealt with, inherent individual variation in sensitivity, body weight and previous treatment.

II. Material and technique.

Urine of pregnancy and anterior lobe extracts. Only one preparation of each was used throughout the experiments; the extracts were kept as dry powders in the cold store, under which conditions they showed no signs of decreasing activity. The amounts required for each test were made into solution immediately before injection. The urine preparation was made by alcohol precipitation of concentrated urine; as will be seen, it was only a crude extract. The anterior lobe extract was made by alcohol precipitation of the pyridine-soluble fraction of acetone-desiccated ox anterior lobes and was kindly supplied by British Drug Houses. As assayed on œstrous rabbits, this extract was much more potent than that from the urine, although it was comparatively crude. Such extracts were purposely chosen for this work because of the greater ease of weighing out the small amounts of the dry powder required for each test.

Preparation of rabbits. The rabbits were obtained from dealers and isolated for 5 weeks before the first test. Many had litters during this time, so that while all should have been in œstrus, some would also have been post-partum at the time of the first test. The interval between consecutive tests on the same group of animals was easily decided. The rabbit returns to œstrus about 3 weeks after a sterile ovulation, and this indicated a suitable interval. At the second and later tests the animals were therefore either in persistent œstrus or else had just come on œstrus after pseudopregnancy, depending on the previous reaction. The use of post-partum animals only, even if advantageous, would be impracticable in large-scale work—either the animals could only be used once, or else 2 months' interval would be required between tests. It is difficult to see what physiological difference there can be between a post-partum rabbit not suckling its young and a post-pseudopregnant one. Although no actual experiments were carried out, there is reason to suppose that the length of the interval, if at least 3 weeks, is immaterial (see p. 344). It remained, however, to ascertain how many times the animals could safely be used at 3-week intervals. So far as the actual operations were concerned, it was found that four to six laparotomies could be performed on the same animal without causing adhesions or other local symptoms in more than a few animals.

The aim was to use not less than twenty animals in each group. For convenience in description, the groups were labelled A, B, C, D, etc., and the successive tests on each group 1, 2, 3, etc. Thus, test B4 means the fourth test on group B, etc. In the absence of any adequate nomenclature for the preparations used, the anterior lobe extracts are referred to for brevity as A.P. extracts and those from the urine as U.P. extracts.

Injection and examination. Injections of the aqueous solution were made into the marginal ear vein, the volume varying between 0·5 and 2 c.c. Next day sterile laparotomies were performed under ether anæsthesia. In groups A, B, D, E, F, G, H, one ovary only was examined if ovulation had occurred. If the first ovary examined was negative, then laparotomy was also performed on the other side. So far as possible laparotomies were alternated between the two sides at consecutive tests. In group C the left side only was opened each time. In each group of rabbits the result of the last test was obtained by autopsy. About 200 autopsies and 900 laparotomies were performed in the course of this work. In a few rare cases where the ovaries of the animal were obviously not in a condition of œstrus the result was discarded. Most of these were due to the presence of only indifferent follicles in the ovary, but four were cases of spontaneous ovulation (as diagnosed by the presence of large recent corpora lutea) during the previous interval. Three of the four followed negative tests, so that there can have been no doubt about the diagnosis. The fourth was found following a positive test 3 weeks before, but there is little room for error as one set of corpora lutea appeared to be only about 1 week old. 157 animals were examined after previous negative tests in the same series of experiments, so that about 2 p.c. of the rabbits ovulated spontaneously during the course of 3 weeks' œstrus.

III. Effect of previous treatment.

The first point requiring investigation is whether injections and laparotomies can be performed indefinitely on an animal without disturbing its sensitivity. The second is whether the result of the previous test has any effect on the response at the next, *i.e.* whether an animal which has just come into œstrus again following an induced pseudo-pregnancy behaves in the same way as one which did not ovulate at the previous test and has therefore been in œstrus 3 weeks. The available data relative to both points is to be found in the histories of groups A, B and C, set out in Table I. Seven consecutive tests at 3-week intervals were made on Group A, varying amounts of U.P. extract being given at

each test. Five tests were made on group B also with varying amounts. Six tests were made on group C, the same amount of extract being given each time. This group also differed from the others since only the left ovary was examined on each occasion.

TABLE I. Induction of ovulation in groups of œstrous rabbits by injection of extract of urine of pregnancy.

Group and test	Date (1933)	Amount given (mg.)	No. of animals	No. ovulating	p.c.* ovulating
Group A, test 1	Jan. 8th	10·0	19	18	95
,, ,, 2	Jan. 29th	5·0	23	13	57
,, ,, 3	Feb. 20th	2·5	23	5	22
,, ,, 4	Mar. 13th	7·5	22	13	59
,, ,, 5	Apr. 4th	7·5	21	8	38
,, ,, 6	Apr. 24th	10·0	21	10	48
,, ,, 7	May 15th	20·0	18	8	44
Group B, test 1	Mar. 9th	5·0	23	8	35
,, ,, 2	Mar. 30th	10·0	21	21	100
,, ,, 3	Apr. 19th	7·5	21	18	86
,, ,, 4	May 11th	10·0	19	11	58
,, ,, 5	June 6th	10·0	20	9	45
Group C, test 1	May 29th	7·5	23	18	78
,, ,, 2	June 19th	7·5	23	18	78
,, ,, 3	July 10th	7·5	22	11	50
,, ,, 4	July 31st	7·5	21	9	43
,, ,, 5	Aug. 21st	7·5	20	6	30
,, ,, 6	Sept. 9th	7·5	21	4	19
Group D, test 1	June 21st	5·0	19	12	63
,, ,, 2	July 11th	2·5	22	1	5
,, ,, 3	Aug. 1st	7·5	22	21	95
Group E, test 1	Oct. 16th	5·0	22	10	45

* To nearest whole number.

Number of times a group may be used. In group A (the first one to be used) the first three tests, at which 10, 5 and 2·5 mg. respectively were given, showed perfectly consistent results (Fig. 1); there was a definite relation between the amount of extract given and the percentage of animals ovulating. At the fourth test, however, 7·5 mg. elicited practically the same response as 5 mg. had done at the second test (*i.e.* 59 p.c. positive). In the next three tests the response of the animals diminished greatly. At the fifth test 7·5 mg. gave only 38 p.c. positive, at the sixth 10 mg. gave 48 p.c. positive, while finally 20 mg. only succeeded in producing 44 p.c. positive.

Group B showed the same decrease in response. The first three tests gave results both consistent among themselves and also with the first three tests on group A. At the fourth and fifth tests, however, 10 mg., which had previously given 100 p.c. positive at the second test, gave only 58 p.c. positive and 45 p.c. positive respectively.

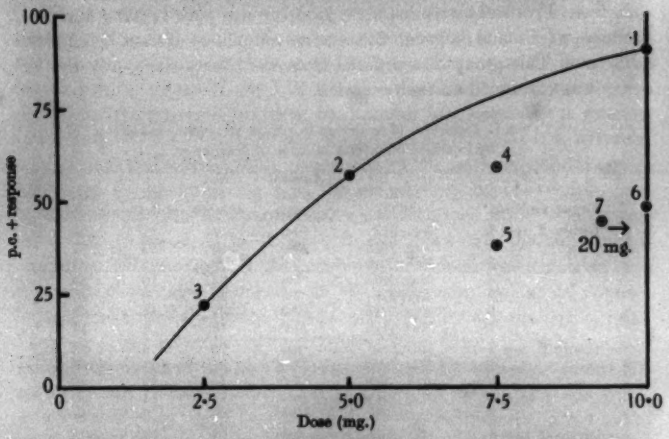


Fig. 1. Response of group A to urine of pregnancy extract, showing nature of curve given by the first three tests, and progressive decrease in sensitivity at fourth to seventh tests.

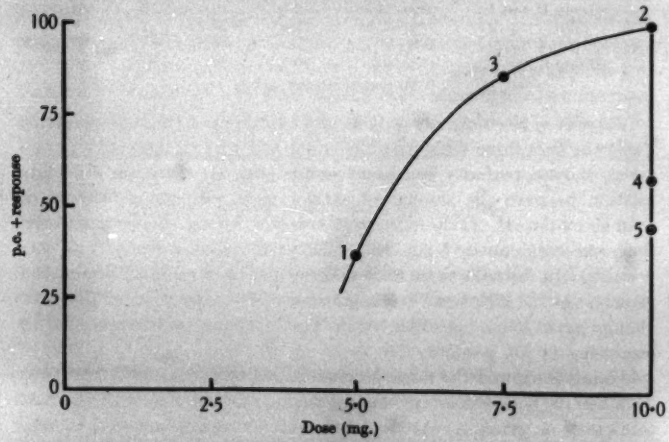


Fig. 2. Response of group B to urine of pregnancy extract, showing nature of curve given by the first three tests, and progressive decrease in sensitivity at fourth and fifth tests.

Three explanations of this falling off in the response of the animals seemed possible: (*a*) that the extract was losing its potency, (*b*) that the animal was becoming affected by the continued laparotomies causing loss of condition, adhesions, trauma or other state adverse to ovarian function, or (*c*) that there was some genuine physiological loss of sensitivity. The first possibility seemed unlikely in view of the fact that the first three tests on group B were done at nearly the same time as the fourth to sixth tests on group A, and there is now adequate proof that deterioration of the extract was not a factor in the falling response. At the time of writing this extract is giving exactly the same results in causing ovulation as it did when the experiments were started more than a year ago.

The second possibility seemed more likely, although even after six operations the animals as a whole had gained rather than lost in weight, and were still showing an abundance of large follicles. Adhesions or trauma might well have restricted the ovarian blood supply without preventing a gradual process such as follicular maturation; on the other hand, no obvious connection had been observed between negative results and the presence of adhesions. It was decided, however, to investigate the question of the effect of handling of the ovary in the following way. Group C was put on experiment and given the same dose of U.P. extract at each test. The dose chosen was 7·5 mg., which had been found to give 86 p.c. positive response in group B and which would therefore make it possible to show a slight increase or a big drop in response at successive tests. The animals of group C were opened on the left side only. This procedure, of course, caused a slight decrease in the observed response as compared with the previous groups (a small proportion of the results recorded as negative by this method might have been positive if both ovaries had been examined), but the aim was to leave the right ovary intact and see whether its response also decreased when there had been no previous handling. The results on group C are given in Table I and show that the successive responses to the same dose progressively fell off after the second test (Fig. 3).

In groups A and B the same ovary was examined on the average about twice in three tests (allowing for one side only being opened where the first ovary gave a positive result), and the earlier falling off in response in group C was thought to be due to the fact that the left ovary was handled at every test. In the first two tests on group C, 78 p.c. were positive. At the sixth test when the animals were killed the left ovaries showed 19 p.c. positive, 11 p.c. lower than the previous result at the

fifth test. The right ovaries, which had not previously been examined at any test and were expected to show the original high response, showed only one more positive out of the twenty-one animals, *i.e.* 24 p.c. The four animals showing ovulation in the left ovary all ovulated in the right ovary; the fifth positive in the right ovary had, of course, been negative in the left ovary. This lack of significant difference in the response of the

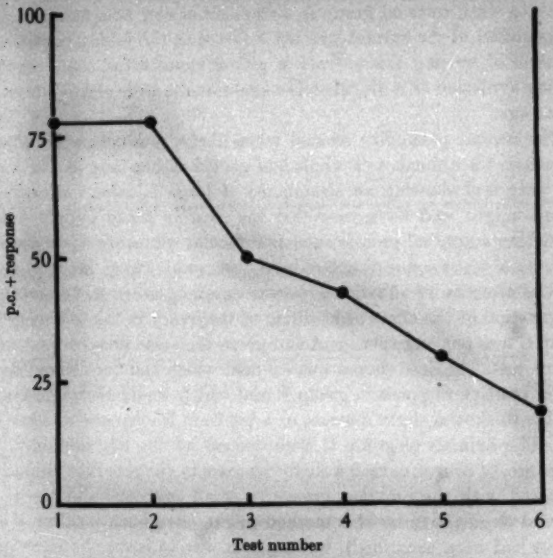


Fig. 3. Response of group C to a constant amount of urine of pregnancy extract on successive occasions, showing the decrease in response.

left ovaries, previously examined five times, compared with that of the right ovaries, not previously handled, seemed to dispose of the possibility that the decrease in response was due to operative interference with the ovary and suggests strongly that there is some physiological decrease in sensitivity when an animal has been used a number of times. Examination was then made of the records which had been kept of the presence of blood follicles in the ovaries when the animals were killed at the last test. Some of the rabbits had a very large number of such follicles. The

distribution of positive and negative results between ovaries with and without blood follicles is shown in Table II.

TABLE II.

	Rabbits with B.F.			Rabbits without B.F.		
	Total	No. ovulating	No. not ovulating	Total	No. ovulating	No. not ovulating
Group A	8	2	6	10	6	4
„ B	6	1	5	14	8	6
„ C	6	1	5	15	4	11

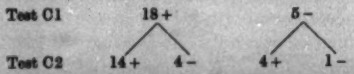
These results may show that an ovary with blood follicles is rather less likely to ovulate than an ovary without or, conversely, that the less sensitive animals are likely to acquire blood follicles, but they cannot explain the great drop in response found at the later tests. Even among animals with no blood follicles the response in group C, for instance, is only 27 p.c. positive (both ovaries) as compared with 24 p.c. for all group C.

Further light was thrown on this decreased response (see p. 344), but no satisfactory explanation has been arrived at. It is clear, however, that without further information, it is not safe to carry out unlimited tests at 3-weekly intervals, and only the first three tests on groups A and B are used for the standardization curve given on p. 351. As a routine, begun immediately after the above results were obtained, an animal is used three times only for assay work. Groups D, E, F, G, H, for instance, as shown in Tables I and IV, were used only three times. No attempt has yet been made to follow the subsequent history of "desensitized" animals.

Effect of previous response. This may be considered either from the standpoint of the group or that of the individual rabbit. As regards the first three tests on each group, our data give no suggestion that a high or a low response at one test causes an abnormally high or low response at the next. Thus test D3 (7·5 mg.), which followed 5 p.c. positive at D2, gave 95 p.c. positive, while B3 (also 7·5 mg.), which followed 100 p.c. positive at B2, gave 86 p.c. positive. Similarly, the highest and the lowest responses on 5·0 mg., B1, 35 p.c. positive, and D1, 63 p.c. positive, were both first tests on the respective groups. Further the two tests on 2·5 mg. (A3 and D2) which gave comparatively divergent results both followed tests on 5 mg., giving very similar results.

The histories of the individual rabbits in the first three tests bear out this superficial impression. Group C offers the best material for examining the point, because no very low or very high percentage of positives was

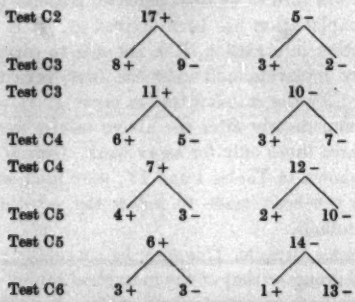
recorded. In the first test, 18 were positive and 5 negative. In test C2 these split up thus:



The ratio of positives to negatives in the animals which had previously been positive or negative was almost exactly the same, showing that a previous positive animal is just as likely to ovulate at the next test as a previously negative animal. The rest of the history of C group is given in Table III.

Owing to a certain number of deaths under anæsthesia, and to some animals being omitted because of bad follicles, etc., the numbers do not always correspond. Thus in test C3, eleven negative animals were available for considering their previous history, but only ten for considering their behaviour at the next test.

TABLE III.



These results show that up to and including the third test the positive and negative results are uninfluenced by the previous response of the animal. After the third test, however, there are signs that the animals acquire a marked tendency, compared with the general reaction of the group, to respond in the same way as they did before. In test C6, three of the four positives are recruited from the six positives at the last test, while thirteen of the fourteen previous negatives remain negative. Thus the increasing number of negatives in successive tests on group C was clearly due to nearly all the negatives from one test remaining negative at the next test and being joined by some of the previous positives. This acquirement of a tendency at the fourth and later tests

admits of no obvious explanation, but is added evidence that not more than three tests should be carried out on each group of animals for assay work.

TABLE IV. Effect of the previous response of individual rabbits in Group C.

	Positive			Negative		
		Next test			Next test	
Test	No.	No. remaining positive	No. changing to negative	No.	No. changing to positive	No. remaining negative
1	18	14 (14·1)	4 (3·9)	5	4 (3·9)	1 (1·1)
2	17	8 (8·5)	9 (8·5)	5	3 (2·5)	2 (2·5)
3	11	6 (4·7)	5 (6·3)	10	3 (4·3)	7 (5·7)
4	7	4 (2·1)	3 (4·9)	12	2 (3·6)	10 (8·4)
5	6	3 (1·1)	3 (4·9)	14	1 (2·7)	13 (11·3)

The numbers in brackets are those which would be expected in the absence of any effect of the previous response.

IV. INFLUENCE OF BODY WEIGHT.

No direct experiments were made of the influence of body weight on the response of the œstrous rabbit to a given dose of ovulation-producing substance. This problem can be investigated in three ways, however, from the existing material:

(*a*) The average body weight of the group can be examined in relation to the general sensitivity of the group.

(*b*) The average weights of the positive and negative animals at each particular test can be compared.

(*c*) The average body weights of different groups given the same dose can be examined in relation to their response.

The three groups receiving U.P. extract had average body weights as follows at the beginning of the experiment: D, 2·9 kg.; A, 3·2 kg.; B, 3·8 kg. The order of sensitivity, on the other hand (see Table VI), was A, D, B, the first being the most sensitive. The three groups receiving A.P. extract had average weights of F, 3·0 kg.; G, 3·1 kg.; H, 3·3 kg.; the order of sensitivity was G, F, H (see p. 353). There is no consistent relation of sensitivity to body weight here.

The average body weights of the positive and negative animals in each test where there was an appreciable number of each is shown in Table V.

In only one test, therefore, B1, is there any suggestion that the animals ovulating tend to be the light ones of the group. Our observations during the course of the experiments show no general tendency for the positives of a group to be the smaller animals.

TABLE V. Average body weights of positive and negative animals at each test.

No. of test	No. of positives	No. of negatives	Av. body wt. (kg.) positives	Av. body wt. (kg.) negatives
A2	13	10	3·1	3·0
A3	5	18	3·0	3·2
B1	8	15	3·5	3·9
D1	12	7	2·8	2·9
E1	10	12	3·5	3·7
F1	8	16	3·0	3·0
F2	16	8	3·0	3·0
G2	13	9	3·3	3·3

We have obtained only one instance of correlation between greater body weight and decreased response. If the results of the four tests at which 5 mg. U.P. extract were given are considered in relation to average body weight of the group, it is found that the percentage response is correlated, at least superficially, with the average body weight. Thus at test D1 the average body weight = 2·9 kg., p.c. positive = 63; at test A2 the figures were 3·2 kg. and 57 p.c. positive, at E1, 3·6 kg. and 45 p.c. positive, and at B1 3·8 kg. and 35 p.c. positive. Against this apparent correlation, however, we find that 10 mg. caused 100 p.c. positive in group B (3·8 kg.) as compared with 95 p.c. in A (3·2 kg.), and 2·5 mg. gave a much better response in group A (3·2 kg.) than in group D (2·9 kg.).

On the whole, it cannot be said that the present material affords any evidence that the body weight of the rabbit, within a reasonable range such as was used, has any constant effect on the ovulation test. This conclusion is in keeping with that arrived at in the assay of œstrin by Marrian and Parkes [1929].

V. RELATION BETWEEN THE PERCENTAGE RESPONSE AND THE NUMBER OF FOLLICLES OVULATING IN EACH ANIMAL.

Throughout the experiments it was noticed that in tests where only a small percentage of animals ovulated, the number of ruptured follicles in each animal was smaller than where a higher percentage reacted. In tests giving a low response the positives comprise animals in which one or two follicles had ovulated, many ripe but unruptured follicles being present. Careful records were kept in groups A, B, D, though these are complicated by the fact that only one ovary was examined where this was sufficient for diagnosis. Analysis of the records failed to show quite as striking a correlation as was expected, but even so there is evidence of

a general tendency. In Fig. 4 the average number of ruptured follicles in one ovary in the positive animals at each test is plotted against the percentage of animals ovulating for each group.

It is noticeable that where practically all animals ovulate, the average number of ruptured follicles is about the same for each group. The decrease in the average number as the percentage of animals ovulating falls is very variable, however, in the three groups. It would appear that groups A and B would ovulate more than two follicles per positive ovary even when the percentage of animals ovulating was low. On the other

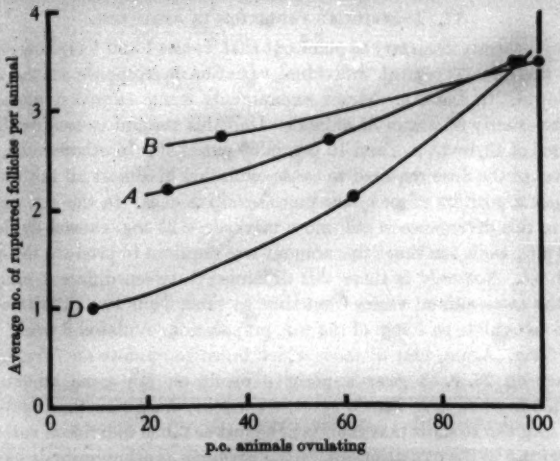


Fig. 4. Relation between the average number of ruptured follicles per positive animal (one ovary) and the percentage of animals ovulating.

hand, there is little trace of an "all-or-none" reaction—*i.e.* no suggestion that all the ripe follicles, if any, ovulate. In the normal animal, however, the reaction after mating is often of this kind; usually the majority of ripe follicles ovulate after copulation. In this connection it is interesting to note that the dose of U.P. extract which causes ovulation in 95–100 p.c. animals only causes an average of about 3·5 follicles per ovary to rupture, a number well below that generally found after copulation in normal rabbits kept under identical conditions, viz. 9–12. This presumably means that the dose of U.P. extract which will induce ovulation in 100 p.c. contains considerably less ovulation-producing activity than is put forth

by the pituitary body after copulation. This fact is clearly associated with the observation [Brambell and Parkes, 1932; McPhail, Parkes and White, 1933] that an appreciable amount of the immediate post-copulation pituitary secretion can be removed by blood replacement without inhibiting ovulation, though the number of follicles rupturing is decreased. There is no doubt that the full number or even an excessive number of follicles can be caused to ovulate by administration of large amounts of U.P. or A.P. extract, and it is possible that some system of assay depending on the number of follicles ovulating could be worked out.

VI. Individual variation in response.

It is scarcely necessary to point out that Tables I and VII show clearly that there is very great individual variation in response to the doses employed. In the U.P. extract experiments 5 mg. caused ovulation in almost exactly 50 p.c. of 87 animals. Half this amount caused ovulation in 6 out of 45, or 13 p.c. and 10 mg. in 39 out of 40. In other words, one-quarter of the dose required to cause ovulation in almost all rabbits will produce a positive effect in the more sensitive ones. In the A.P. experiments, this divergence is still more marked. 0·25 mg. caused ovulation in 10 p.c., while ten times this amount was required to produce the effect in 95 p.c. Not only is there this difference between different animals, but the same animal varies from time to time. Thus two rabbits which failed to ovulate on 5 mg. of the U.P. preparation, ovulated 3 weeks later on 2·5 mg. Again, four of those which failed to ovulate on 7·5 mg. U.P. extract on 29. v. 33 gave a positive result on the same amount on 19. vi. 33, while four others reacted conversely. No further examples need be given to show that rabbits have just as much individual variation in response to the ovulation-producing hormone as other animals have in response to drugs and hormones, and therefore that any such concept as the "minimum effective dose" is fundamentally unsound unless "effective" is very carefully defined.

VII. Group variation in response to U.P. extract and construction of standardization curve.

We have obtained but few data regarding the variation in response of the same group to the same dose at different times. In group C the first two tests with 7·5 mg. U.P. extract happened to give identical results; at the third test there was a considerable drop, but by then this particular group appeared to have begun the progressive decline in response which continued until the animals were killed.

The problem of variation in response in different groups to the same dose is intimately connected with the position and slope of the response curve obtained for any particular group. Of the three groups injected with U.P. extract, group A (Fig. 1) obviously has a flatter curve than either of the other two groups (Figs. 2 and 5), *i.e.* the increase in percentage response for a given increase in dosage is less. Of the other two groups, B has a slightly steeper curve than D (Fig. 5). Dr J. H. Gaddum has examined these curves by the method outlined by him in a recent report [1933]. The characteristic curves obtained by plotting the normal

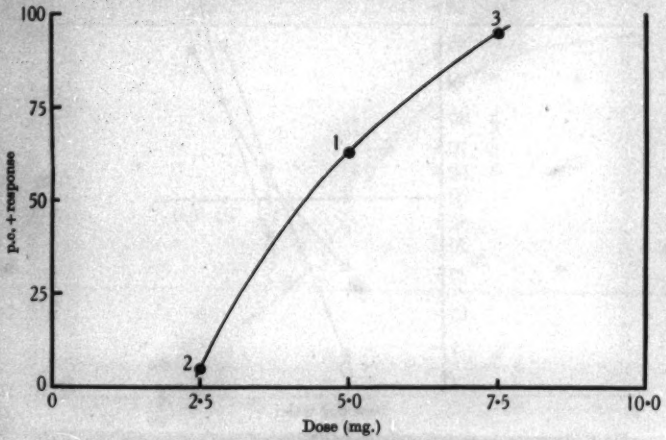


Fig. 5. Response curve for group D receiving urine of pregnancy extract. Three tests only were made on this group.

equivalent deviation against $\log_{10}$ dose are close approximations to straight lines, the theoretical expectation (Fig. 6). The relevant data are shown in Table VI.

Dr Gaddum concludes that while the dose required to produce ovulation in 50 p.c. animals is fairly constant for the three groups, the slope is surprisingly variable. This fact is of interest, in that the percentage response curves for other substances and other animals tend to shift their horizontal position from time to time and from one group to another, while retaining a constant slope. The practical application of the variation of slope in these curves is that many more animals per

group are required to attain a given accuracy of assay with flat curves, and that one batch of animals may not be entirely comparable with another.

It is improbable that these variations in slope are due to chance, but it is difficult to distinguish the exact factors at work. The variation has no appearance of being seasonal. The flattest and steepest curves were

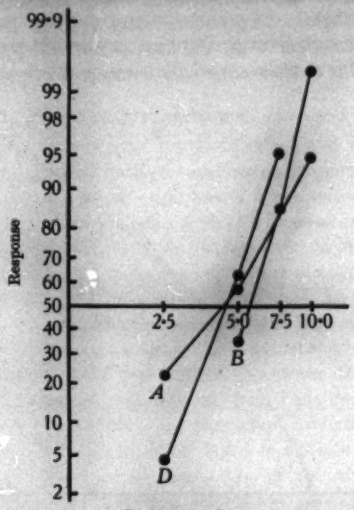


Fig. 6. Response (as normal equivalent deviation) plotted against dose ($\log_{10}$ scale) for groups A, B, D.

TABLE VI.

Group	Date	O.D. 50 (mg.)	b
A	Jan.–Feb.	4·2	3·8
B	March–April	5·6	9·3
D	June–Aug.	4·45	7·0

O.D. 50 = dose required to produce ovulation in 50 p.c.
b = slope of the curve [see Gaddum, 1933] the greater the number the steeper the curve.

made early in the year, and the intermediate one in midsummer. Similarly, such variation as was found in the O.D. 50 (including group E, where O.D. 50 would be rather over 5 mg.) showed no regular correlation with season, although the four points were well distributed throughout the year. In any case, seasonal variation would not be expected in an animal

with no definite anœstrus. Environmental temperature has been found to affect the response of animals to certain substances [see Gaddum, 1933], but the rabbits were kept in a fairly constant environmental temperature.

Body weight is a possible factor, although the data given in Section IV give very little suggestion that the actual body weights affected the response. In any case, to affect the slope of the curve, as distinct from its position, variation in scatter of the body weights in the groups would be required. At the beginning of the experiment the average body

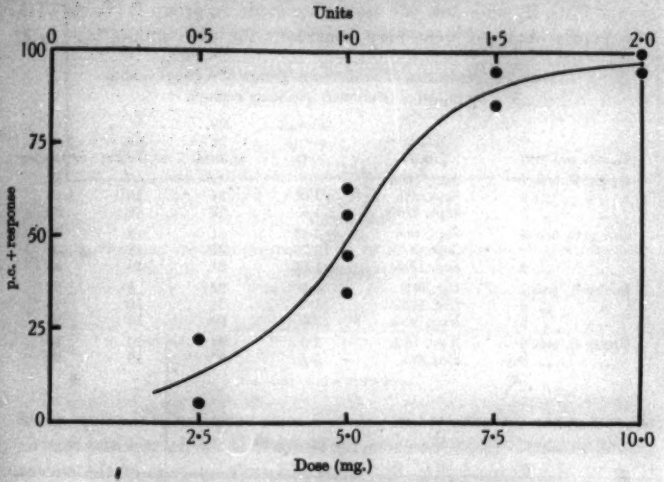


Fig. 7. Standardization curve for urine of pregnancy extract.

weights of the groups, with their standard deviations, were as follows: A, $3{\cdot}2 \pm 0{\cdot}35$ kg.; B, $3{\cdot}8 \pm 0{\cdot}37$ kg.; D, $2{\cdot}9 \pm 0{\cdot}58$ kg. Thus there is no great probability that the variation in body weight in the group is connected with the variations in slope. Another factor known to affect the slopes of such curves, homo- and heterozygosity of the animals, is unlikely to be of importance here.

In view of the rather different results given by the three groups, and of the undesirability of trying to obtain more than three points from one group, it remains to decide the best form of standardization curve to adopt. Dr Gaddum recommends that a curve should be used corre-

sponding to the average of the figures given in Table VI, *i.e.* O.D. 50 = 5 mg. and $b = 6{\cdot}7$. Actually, a close approximation to such a curve is obtained by fitting a curve graphically to the ten points available (three from groups A, B, D and one from E). Such a curve is shown in Fig. 7.

VIII. Group variation in response to anterior lobe extract and construction of standardization curve.

Three tests with anterior lobe extract were carried out on each of groups F, G, H, while two odd tests were made on group E (Table VII). The points obtained were very consistent for each group, but as in

Table VII. Induction of ovulation in groups of œstrous rabbits by injection of anterior pituitary extract.

Group and test	Date (1933)	Amount given mg.	No. of animals	No. ovulating	p.c.* ovulating
Group F, test 1	Aug. 15th	0·5	24	8	33
" " 2	Sept. 5th	1·0	24	16	67
" " 3	Sept. 26th	1·5	23	19	83
Group G, test 1	Sept. 9th	0·25	21	2	10
" " 2	Sept. 27th	0·75	22	13	59
" " 3	Oct. 18th	1·25	21	18	86
Group H, test 1	Oct. 9th	0·5	22	6	27
" " 2	Oct. 30th	1·5	21	15	71
" " 3	Nov. 20th	2·5	20	19	95
Group E, test 2	Nov. 15th	2·0	24	20	83
" " 3	Dec. 6th	2·25	21	19	90

* To nearest whole number.

the case of the U.P. extract tests, there is some variation in the slope of the curves obtained. Thus, the curve for H group is the flattest and that for G group the steepest (Fig. 8). Dr Gaddum's analysis of the curves, however, (Fig. 9) shows that there is much less variation in slope than in the curves for U.P. extract; the index b is between 3 and 4 for all three curves (E group tests are omitted, since there are only two). To this extent, therefore, the A.P. extract curves are more satisfactory than those of the U.P. extract. On the other hand, the A.P. extract curves are less satisfactory in that their slope is only about as great as that of the flattest of the U.P. extract curves (A group). Also, while the slope is fairly constant, their horizontal position is slightly more variable. Thus O.D. 50 is 0·7, 0·64 and 0·85 mg. for groups F, G, H respectively. On the whole, therefore, the two sets of curves may be considered about equally satisfactory. The general remarks made in Section VII apply equally to the A.P. extract curves.

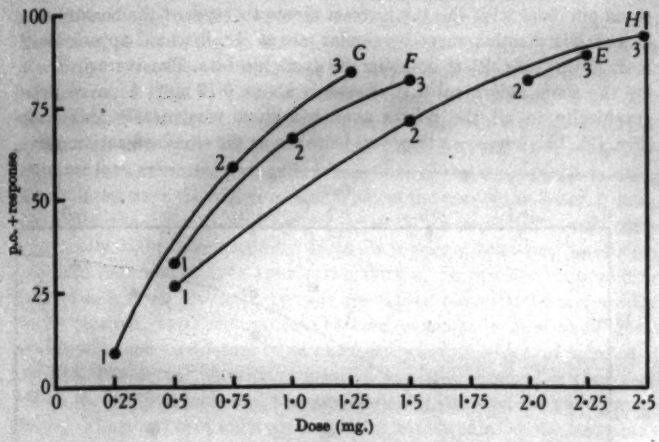


Fig. 8. Response curve for groups F, G, H, receiving anterior lobe extract, showing general nature.

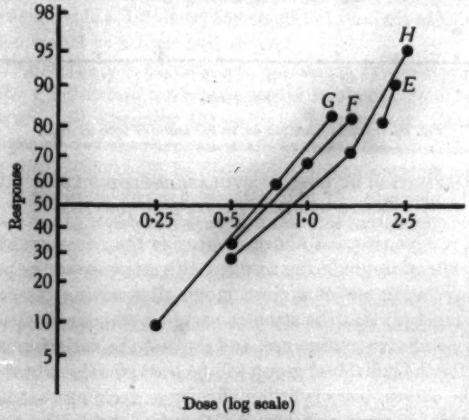


Fig. 9. Response (as normal equivalent deviation) plotted against dose ($\log_{10}$ scale) for groups F, G, H.

The general similarity of the results with anterior lobe extract to those obtained with the U.P. extract seems to warrant the construction of a standardization curve by similar means, *i.e.* it would appear legitimate in this case also to combine the available data. The average O.D. 50 for the three individual A.P. curves is about 0·73 mg. A curve fitted graphically to all the points available gives very nearly this value (Fig. 10). This curve can therefore be taken as the standardization curve.

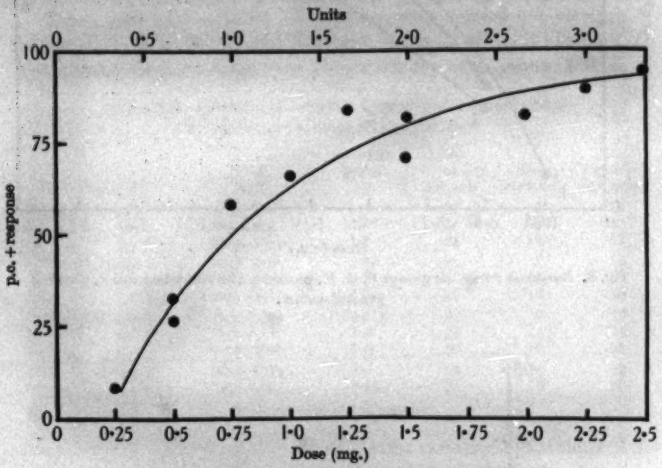


Fig. 10. Standardization curve for anterior lobe extract.

IX. Definition of unit of ovulation-producing activity and use of standardization curves.

All the results recorded above emphasize the necessity of defining the unit of ovulation-producing activity with reference to the percentage of rabbits ovulating out of a given group all receiving the same dose. Bearing in mind (*a*) that the steepest part of a standardization curve is the most accurate for routine use, and (*b*) that the variation in slope of the curves for the individual group will be least effective about the mid-points of the curves, there is little difficulty in deciding that the 50 p.c. ovulation point is most suitable. We therefore define the unit as the ovulation-producing activity contained in the amount of a preparation

required to cause ovulation in 50 p.c. of a group of œstrous rabbits, the substance being administered as a single intravenous injection. The horizontal scale of mg. in the two standardization curves can thus be converted to scales of units (see the top abscissæ). Thus in the U.P. extract curve (Fig. 7) 5 mg. corresponds to 1 unit, 2·5 mg. to 0·5 unit and so on. With the A.P. extract curve, 0·75 mg. corresponds to 50 p.c. ovulations or 1 unit, 1·5 mg. to 2 units, and 2·25 mg. to 3 units. The anterior lobe extract curve differs from that of the U.P. extract in being much flatter over the higher ranges. Thus in the case of the latter, 2 units, *i.e.* twice the amount required to cause 50 p.c. ovulations, will cause practically 100 p.c. ovulations. In the A.P. curve, however, 3 units are required before the curve approaches 100 p.c. In practice such a curve is used as follows: Preliminary tests are carried out with the preparation to be assayed, and information obtained to enable a dose to be given which will cause ovulation in as nearly as possible 50 p.c. of a batch of rabbits. The actual percentage positive response obtained is then read off on the vertical scale and the corresponding point on the curve ascertained. The number of units corresponding to this point on the curve may be read off on the abscissa. Thus if 1 mg. of an unknown U.P. preparation produced ovulation in 30 p.c. of a batch of rabbits this amount would be found to correspond to 0·8 units, *i.e.* the unit of this particular preparation would be 1·25 mg. Similarly if 0·5 mg. of an unknown A.P. extract gave 60 p.c. ovulations (*i.e.* 1·2 units) the weight of the unit of this particular preparation would be a little over 0·4 mg.

The error of the U.P. extract test, calculated by drawing lines corresponding to ± 2 standard deviations, would be about 25 p.c., with groups of twenty animals, assuming the curve to be constant. Changes in the slope and position of the curve naturally increase the error. Error due to change in the O.D. 50 could be diminished by the simultaneous use of a standard preparation on each occasion, but the observed alteration in these experiments is not sufficient to make this necessary. Error due to change in the slope could be controlled by testing two different doses on each occasion, but in practice it would appear easier to carry out sufficient preliminary tests to be able to give such a dose to the group that between 30–70 p.c. positives are obtained, over which range variation in slope of the curve, with a constant O.D. 50, will cause least error. In the case of the A.P. extract curve its increasing flatness in the upper ranges makes it most desirable to restrict use to the 30–70 p.c. range.

As a final practical point it is necessary to consider what number of animals should be used. Standard curves of this kind should be built

by each set of workers for their own use, since curves obtained in one laboratory would not necessarily serve in another; and it is clearly desirable to use as many animals as possible in the construction of the original curve. In the foregoing experiments, groups of twenty were chosen as being the maximum which it seemed possible to handle. Having obtained the standardization curve, the number of animals which can be used for each test has to be determined as a compromise between desirability and convenience, and if any large number of tests are to be made it would seem that, after the preliminary trials, the use of batches of ten œstrous rabbits will be about the best that can be managed under ordinary laboratory conditions. In this connection it is of importance to remember that, other things being equal, the accuracy of a test varies only as the square root of the number of animals used. Thus a test on twenty animals, so far as mere numbers go, is only 50 p.c. more accurate than one on ten animals.

X. Relation of the rabbit ovulation test to that on the immature mouse or rat.

As we have emphasized above, the rabbit ovulation test is a criterion of the ovulation-producing activity of an extract; the test on the immature mouse or rat, depending on the reaction observed, is a criterion of the power of an extract to cause (*a*) œstrin production by the immature ovary, (*b*) the formation of lutein tissue, or (*c*) an increase in the weight of the ovary due to enlargement of follicles or formation of blood follicles, or atretic or true corpora lutea. In other words, except where corpora lutea are used as the test object, the test on the immature mouse depends on different activities of an extract from the rabbit ovulation test. Even the production of lutein tissue in the immature animal is dependent on the extract possessing follicle-growing activity, which the rabbit ovulation test is probably not. There is very great doubt whether all the ovary-stimulating effects are caused by one substance, and it is evident therefore that the immature mouse test probably depends on different or at least additional substances in the extract to the rabbit ovulation test. To compare the mouse (or rat) unit and the rabbit unit of an extract is therefore fundamentally unsound. An analogy would be to make an alcoholic extract of whole ovaries containing corpora lutea and compare the amount required to cornify the vagina of the mouse with that required to cause pseudo-pregnant proliferation of the uterus of the rabbit! From this point of view there seems to be no sufficient reason for concluding that the corresponding U.P. and A.P. principles are different substances

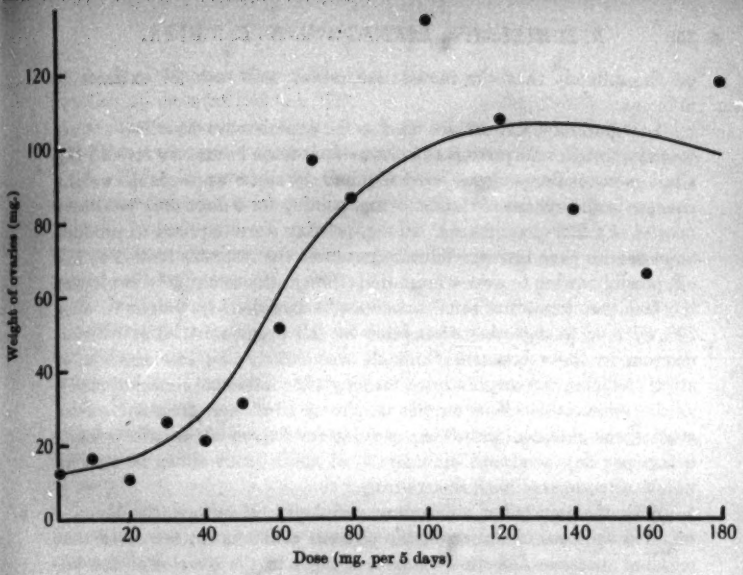


Fig. 11. Effect of the anterior lobe extract on the weight of the immature rat ovary.

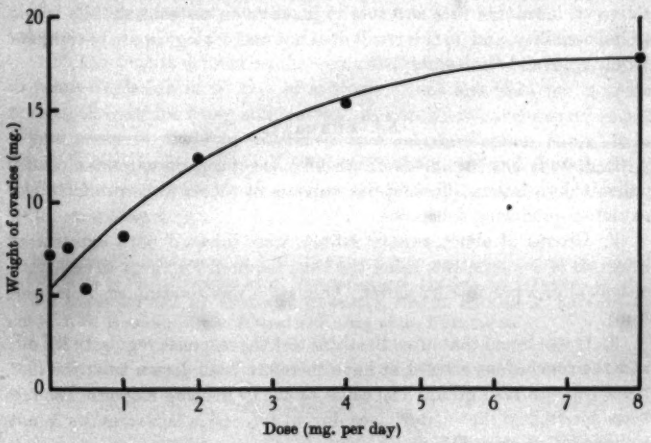


Fig. 12. Effect of the anterior lobe extract on the weight of the adult mouse ovary.

on the grounds that the mouse unit/rabbit unit ratio of extracts is different.

As regards the A.P. extract used in the experiments described above, we have certain information as to its action on the immature rat and the adult mouse. Large doses were required to cause appreciable weight changes in the ovaries of the rat; 8 mg. per day for 5 days only produced ovaries of a little over 20 mg. 20 mg. per day were required to produce large ovaries over 120 mg. With larger doses the response tended to fall off, possibly owing to over-stimulation (though this is unlikely in view of the fact that immature rat ovaries can be increased in weight to over 200 mg.) or to defective absorption of the preparation. The ovarian reaction in these immature animals was largely the luteinization of atretic follicles, the corpora lutea forming after follicular stimulation.

An appreciable effect on the weight of adult non-pregnant mouse ovaries was obtained with 3 mg. per day for 5 days of the A.P. extract; 6 mg. per day produced an increase of nearly four times in ovarian weight as compared with the controls.

With the particular A.P. extract employed, therefore, the amount required to cause ovulation in the œstrous rabbit is far less than that required to cause detectable weight changes in the ovaries of the immature rat or adult mouse. We emphasize again, however, that (*a*) this relation might not hold for another A.P. extract; a preparation highly active on immature rats and totally inactive on œstrous rabbits is not an impossibility, and (*b*) this result does not make it legitimate to compare rabbit units and "rat units."

XI. Summary.

1. A study has been made of the influence of certain variables, chiefly individual variation, affecting the response of the œstrous rabbit to the ovulation-producing substance.

2. Groups of about twenty rabbits were injected with extracts at intervals of 3 weeks, this being the time required for them to return to œstrus after a previous ovulation. Ovulation was determined by laparotomy.

3. It was found that after the third test the response regularly fell off, and the conclusions arrived at have therefore been drawn from the first three tests on each group. The cause of the decreasing response has not been determined, but disturbance due to successive laparotomies is not responsible (Section III).

4. The response of an individual at one test does not influence the reaction at the next (Section III).

5. Within reasonable limits, the body weight of the animal does not appear to exert any consistent influence on the response (Section IV).

6. There is some correlation between the percentage of animals ovulating and the average number of follicles ovulating per positive animal. Doses which will cause ovulation in practically 100 p.c. do not, however, cause the rupture of as many follicles as is usually found in the mated rabbit (Section V).

7. Individual variation in response was found to be very considerable. 10 mg. urine of pregnancy extract caused ovulation in practically 100 p.c., but 2·5 mg. caused the response in 13 p.c. With anterior lobe extract the range was even greater (Section VI).

8. Some variation in the response of groups of twenty animals was found, both in the steepness and in the horizontal position of the percentage response curve (Figs. 1, 2, 5). With the urine of pregnancy extract the variation in the dose required to cause 50 p.c. response in the different groups was not great, but appreciable variation in the slope of the curve was found. This variation, however, did not appear to be connected with any obvious factor (Section VII). With the A.P. extract, the variation in the amount required to cause 50 p.c. ovulation was greater, but that in the slope of the curve was less (Section VIII).

9. Standardization curves were constructed for both types of extracts.

10. The unit is defined as the ovulation-producing activity required to cause ovulation in 50 p.c. of a group of not less than ten œstrous rabbits, the extract being administered as a single intravenous injection. Where assay is made by reference to a standardization curve, it is desirable that larger groups of animals should be used to construct the original curve and that such a curve should only be used over the 30–70 p.c. response range.

We are greatly indebted to Dr J. H. Gaddum for the analysis of the response curves and for much of the information about them incorporated in Sections VIII and IX.

Our thanks are also due to Miss R. Deanesly, Miss M. Hill, Dr M. K. McPhail and Mr I. W. Rowlands, who assisted with many of the laparotomies.

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THE ABSOLUTE VALUE OF THE ISOMETRIC HEAT COEFFICIENT DETERMINED BY MEANS OF HIGH-FREQUENCY CALIBRATION OF THE LIVING MUSCLE.

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(*Received March* 16, 1934.)

The absolute value of the isometric heat coefficient in a muscle twitch, namely,

$$\frac{Tl}{H} = \frac{\text{tension developed (g.wt.)} \times \text{muscle length (cm.)}}{\text{initial heat produced (g.cm.)}},$$

is the simplest expression of the connection between thermal and mechanical response in muscular contraction. From Meyerhof's original data on the caloric quotient and the isometric coefficient of lactic acid Hill [1928] calculated for the isometric heat coefficient a value of 6·14 in close agreement with the mean value determined directly. This value was constant during a series of twitches, diminishing only when fatigue started, although a decrease might have been expected from the start owing to the breakdown of phosphocreatine in the early stages of activity. When Lundsgaard discovered the inhibition of lactic acid formation by iodoacetic acid it was shown by Fischer [1931], Hartree [1931], Meyerhof, Lundsgaard and Blaschko [1931] that the magnitude of Tl/H is not altered when the muscle is poisoned. Recently, Meyerhof, Gemmill and Benetato [1933] compared the isometric oxygen coefficient

$$K_{mO_2} = \frac{\text{tension (g.wt.)} \times \text{muscle length (cm.)}}{\text{oxygen consumed (g.)}}$$

for the two cases (*a*) directly observed in normal (and poisoned) sartorii, and (*b*) calculated from the isometric heat coefficient. If, per g. of oxygen consumed in carbohydrate combustion, 151×10^6 g.cm. of heat is liberated, and if, according to Hill, the initial heat is on the average half

the total aerobic heat, the relation should exist $K_{mO_2} = 151 \times 10^6 \times Tl/2H$. Since the authors found K_{mO_2} to be about equal to 700×10^6 in normal muscles, the isometric heat coefficient would need to be 9·4; the average minimum value of K_{mO_2} being 667×10^6 would correspond to $Tl/H = 8{\cdot}9$.

In Meyerhof's laboratory, Blaschko [1933], using direct myothermic methods, found a value of Tl/H of 7 to 8 in the sartorius muscle of *Rana temporaria*.

Because of these various discrepancies Prof. A. V. Hill suggested a redetermination of Tl/H under optimal conditions, and in particular the calibration of the living muscle with high-frequency currents in order to find out if the results differ from those obtained with calibration of the dead muscle as employed in all previous methods.

Two methods previously employed on dead muscles were compared with one another and with the high frequency calibration.

(1) *Calibration by condenser discharges.* This method, suggested by Bozler [1931] and worked out in detail by Hill [1931], is based upon the principle of heating the muscle by means of the known energy of a condenser discharge. If the wires and electrodes have negligible resistance all the energy is set free as heat in the muscle. Taking C in microfarads and V in volts, the energy of a charged condenser is $5CV^2$ ergs. It is sufficient, therefore, to measure V exactly and to read the deflection of the galvanometer connected to the muscle thermopile when the condenser is discharged through the muscle. A mica condenser of 0·5 or 0·8 μF was used, calibrated against a standard within 0·001 μF and charged to about 60 volts. The potential was read on an accurate high-resistance voltmeter. It is possible to use discharges only, or charge and discharge; the latter would avoid any results of polarization at the electrodes, which, however, has not much effect when so high a potential is used (see Fig. 1).

(2) *Calibration by audiofrequency currents* [Hill, 1928]. A sinusoidal alternating current is sent through the muscle for a certain time t and the deflection M of the galvanometer connected to the muscle thermopile is read (see Fig. 2). In series with the muscle is a thin wire wound non-inductively round the hot junctions of a thermopile. A galvanometer connected to the hot-wire thermopile gives a deflection H. When a resistance is substituted for the muscle in series with the hot wire the muscle resistance R is found when the hot-wire thermopile again gives a deflection H. Finally, the intensity of the alternating current is measured by comparison with a direct current of potential e, giving a deflection h with the hot wire for a resistance r in the circuit. From these figures it

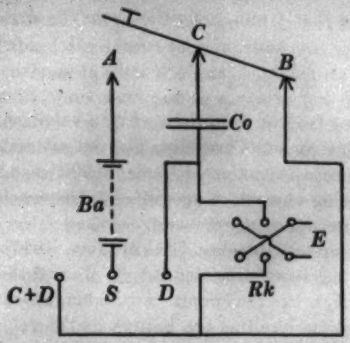

Fig. 1. Connections for stimulating and calibrating with condenser charges and discharges, or with discharges only. *ACB*, morse key; *Ba*, battery; *Co*, condenser; *Rk*, reversing key; *E*, electrodes. If *S* is connected to *C* + *D* the charge and the discharge pass through the muscle; if *S* is connected to *D* the discharge only is applied.

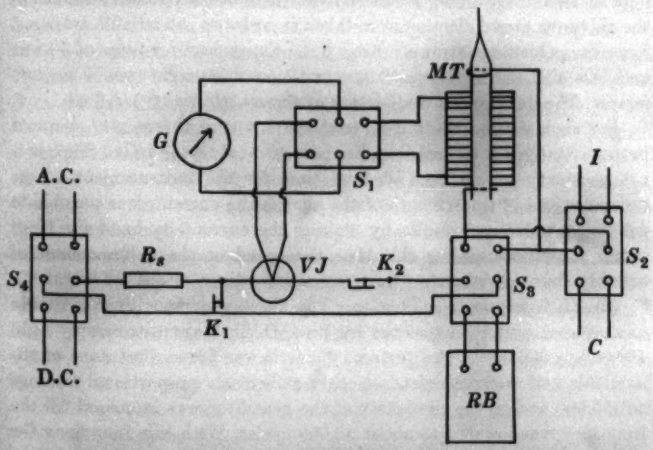

Fig. 2. Connections for calibration with alternating currents or condenser discharges. A.C. high frequency or audiofrequency; D.C. accumulator; R_s, 500 or 1000 ω; *VJ*, vacuo-thermojunction; K_1, short-circuit key; K_2, in-circuit key of revolving contact breaker; *MT*, muscle thermopile with muscle; *G*, galvanometer; *RB*, non-reactive resistance box; *C*, to condenser; *I*, to induction coil; S_1, S_2, S_3, S_4 double-pole switches.

can be calculated that 1 mm. deflection from the muscle thermopile is equivalent to

$$\frac{HR}{M} \times \frac{e^{\alpha t}}{r^{\alpha t}} \times 1{\cdot}019 \times 10^4 \text{ g.cm.}$$

The alternating current was produced by a valve generator type A of the Cambridge Instrument Company. The output coil (500 turns) was shunted by a non-reactive potential divider of 2000ω supplying a suitable intensity of heating current. Two different frequencies of about 300 and 3000 cycles respectively were used.

A revolving contact breaker [Parkinson, 1926] determined the time t. Opening the short-circuiting key K_1 allowed the current to flow, opening the key K_2 broke the circuit. A suitable resistance R, was introduced to avoid short-circuiting the battery or the valve generator with the first key. The contact breaker was tested with two signal magnets on a smoked drum, worked each by one of the keys, and the average of four to eight records was taken. It was found that with short intervals the time varied as much as 5 p.c., even if the spring of the clockwork was kept at medium tension. In every case, therefore, six to ten readings of the galvanometer deflection were taken in order to get reliable averages. Since the percentage error is greater if the time is less, for times of 0·1 sec. and less a Helmholtz pendulum with counterweight would be preferable. The duration of the heating of the muscle was 0·1–0·3 sec.

For such a time the muscle and the hot-wire thermopile operated proportionally, *i.e.* the reading was proportional to the heat. Since it is not necessary to use such a short time for the measurement of the resistance and of the strength of the alternating current, it is possible to get quick and exact results by making the current by hand for 10 or better for 20 sec. After this time the speed of the galvanometer, if suitably shunted, is so slow that accurate readings can easily be taken.

(3) *High-frequency calibration.* The valve generator used for these measurements was constructed by Prof. D. T. Harris to supply from 100,000 to 300,000 cycles per sec. Since it was found that none of the available non-reactive resistances gave sufficiently proportional readings at 100,000 cycles, the condenser of the generator was increased till the frequency was lowered to about 45,000 cycles. With this frequency the resistance was reliable. The muscles did not twitch and were not in any way altered when this high-frequency current, of a suitable strength for heating them (about 1·0 milliampere), was passed through them. The general procedure was the same as for the case of the audiofrequency current. Instead, however, of the hot-wire thermopile a vacuo-junction

(Cambridge Instrument Company) was used. (In the later experiments summarized in Table I the same vacuo-junction was used also with the audiofrequency currents.) Since the couple resistance was only about 9 ohms a further 25 ohms were put in series to get the galvanometer critically damped. The vacuo-junction gave deflections proportional to the heat for a duration of heating of 0·1–0·3 sec., and no appreciable difference for opposite directions of the calibrating direct current. Using the contact breaker with high-frequency currents the circuit has to be well insulated in order to avoid any creeping of the galvanometer if the shunt is closed or only one side of the circuit is connected. It might be useful to break both lines simultaneously by means of two opening contacts.

All the readings and calculations can be made in the same way as for audiofrequency.

(4) *Experimental details.* The muscle thermopile made by Mr A. C. Downing and used throughout the experiments was of the constantan-iron type with soldered junctions and had 63 couples and a resistance of about 30 ohms. It was connected to a moving coil galvanometer (also built by Mr Downing) having an internal resistance of 40 ohms, an undamped period of 2·6 sec., critical damping at about 40 ohms external resistance, and a sensitivity of $7{\cdot}42 \times 10^{-10}$ amp. per mm. deflection at 3 m. distance. The single twitch of a double sartorius muscle on the thermopile gave a deflection of 120–180 mm. Medium-sized male English frogs (*R. temp.*) in good condition were used. The weight of the two muscles was about 200 mg. The weight of the part between the electrodes, distant 20·3 mm. from one another, was about 120 mg. The muscles were fixed to an isometric lever by a straight fine wire. To prevent shortening of the muscle as completely as possible it is necessary to have an inextensible connection and a lever made with a strong spring; otherwise the value of Tl/H will be considerably reduced. Moreover, the "optimum" length is required, corresponding usually to 3–4 g. initial tension, and was found by trial in every case [see Hill, 1931]. Before stimulation the muscles were soaked on the thermopile in oxygenated Ringer's fluid containing about 8 mg. P/100 c.c. (*p*H 7·2), which was replaced after 2 hours by oxygen.

(5) *Results.* To ensure sufficient recovery, intervals of 2–3 min. were allowed between successive twitches. In the first series of experiments the muscles were stimulated by charge and discharge of a condenser made as rapidly as possible by means of a Morse key. It was seen that in such a double twitch, as compared with a single one, the heat produced

was increased more than the tension developed. For example, T/H was found in five different experiments to have the following values:

Single shock	Double shock
4·56	2·96
3·71	2·40
3·85	2·13
4·15	2·26
3·92	3·04
Mean 4·04	2·56

The value for the single shock is about 50 p.c. greater than for the double shock. Since the two shocks were in opposite directions it was thought that the same stimulus, even if maximal at the upper electrode near the entry of the nerve, would be submaximal at the nerve-free pelvic end. Single discharges, therefore, in both directions, *i.e.* cathode near the nerve entry or at the pelvic end, were compared; the results were unexpected. If the muscle was stimulated at the nerve-free end the tension developed was generally much less, but the heat produced was usually much larger, than if stimulated at the tibial end. Three different muscles gave the following figures for T/H:

Tibial part	Pelvic end
3·90	2·66
3·94	2·84
3·71	3·04
Mean 3·85	2·85

The value for stimulation near the nerve entry is about 35 p.c. greater than for stimulation at the pelvic end. The magnitude, however, of the heat showed that the stimulus at the pelvic end was able to excite the same number of fibres as the stimulus applied in the tibial part.

Why, then, is the mechanical effect reduced? There appears to be no record of any observations made under the precise conditions of these experiments. Bethe and Happel [1923] analysed the movements of curarized muscles working on an isotonic lever. Adrian [1923], with normal sartorii and an isometric lever, changed the strength of the current but apparently not its direction. Rushton [1932*a*], recording tension-time curves with his ballistic lever, showed the deflection after α-stimulation of the muscle to be less than that after stimulation of the nerve; later on [1932*b*] he found a slight delay of the maximum and of the relaxation of the isometric twitch after α-stimulation, but did not deal with the absolute size.

An explanation may probably be given on the following lines: (*a*) if the muscle be stimulated near the so-called motor point, the nervous

substance is excited and the impulse spreads rapidly throughout the nerve fibres and produces an almost simultaneous start of the contraction in widely different parts of the muscle; whereas the contraction excited at the pelvic end travels as a slow wave along the muscle. If this is the case, then (*b*) the contraction of the stronger pelvic part when stimulation is at that end stretches the weaker tibial part not yet in action, and the first part of the muscle excited is already relaxing before the distant parts have reached their maximum tension. This phenomenon was seen by Hartree [1933] even if the muscle, fixed isometrically to two rigid electrodes, was stimulated at the usual point. As an analogy the slow and small contraction of the cooled frog's heart becomes quick and large if the natural impulse, slowly travelling its complicated path, is replaced by an induction shock suitably applied to excite the whole syncytium at once [Rosenberg, 1920].

The heat excess in direct stimulation would be due, according to Fenn [1924] and Azuma [1924], to the shortening of some parts of the fibres in consequence of the stretching of other parts.

Consider the following experiment:

		T (g.)	H (g.cm.)	T/H
Single shock,	tibial	57·7	15·57	3·71
,, ,,	pelvic	56·5	18·55	3·04
Sum		114·2	34·12	
Double shock		95·9	39·86	2·40

This is almost the only experiment giving nearly the same tension for both directions of the current; it is therefore the more convincing in showing the deficit of tension and the excess of heat for the case of the double shock, as compared with the sum of the single shocks.

It was not the purpose of the present work to deal with the case of a double shock, so that after the anomaly referred to above had been disposed of single discharges only were employed with the cathode near the nerve entrance in the tibial third. After, say, twenty twitches, calibration with high-frequency currents was interposed, followed by several twitches to test the condition of the muscle after the artificial heating. The muscle was then killed in nitrogen by means of strong induction shocks, in order to allow comparison of different types of calibration of the dead muscle. In conjunction with the condenser calibration the deflection produced by the energy of the maximal stimulus applied to the living muscle was also determined. It was of the order of 1 p.c. of the initial heat.

The first series of observations contained a comparison of the con-

denser and the audiofrequency method on dead muscles, not previously investigated systematically. The result of seven experiments was that the alternating current of audiofrequency gave a calibration number which was on the average 0·6 p.c. less than the condenser discharge, varying from −2·45 to +0·8 p.c. There was no significant difference between the higher and the lower rates of audiofrequency. In some cases it was found that if the muscles were kept in nitrogen and the readings repeated after a few hours the deflection due to the condenser discharge was diminished and the calibration constant consequently increased, while the alternating current gave the same value as before. The deviation amounted to only a few p.c. A reasonable explanation seems to be that if the resistance at the contact between muscles and upper electrode increased gradually, the rise of temperature due to the condenser discharge would be higher at this part than in the main part lying on the hot junctions. If the muscles were kept in Ringer's solution after killing, the calibration constant was increased by all methods, owing to the swelling of the muscle.

TABLE I. Single twitch of double sartorius. Columns I = g.cm. initial heat; columns II = Tl/H.

	High frequency				Audio-frequency		Condenser discharge	
	Living		Dead		Dead		Dead	
Date 1934	I	II	I	II	I	II	I	II
29. i.	18·11	8·60	18·60	8·39	18·37	8·50	19·02	8·19
30. i.	23·55	7·90	23·74	7·84	23·06	8·06	25·00	7·45
2. ii.	18·65	8·69	19·12	8·47	19·92	8·14	20·08	8·07
5. ii.	18·52	7·98	18·96	7·79	18·86	7·83	19·25	7·67
6. ii.	20·38	8·81	19·25	9·33	19·56	9·19	19·89	9·03
7. ii.	17·13	8·36	16·71	8·56	17·26	8·29	17·33	8·26
8. ii.	15·96	9·25	15·20	9·70	15·04	9·81	15·18	9·73
9. ii.	15·57	7·52	15·14	7·74	15·61	7·50	15·86	7·38
12. ii.	15·56	7·70	13·38	8·95	13·78	8·70	13·30	9·00
13. ii.	16·58	7·81	16·10	8·05	15·90	8·15	16·36	7·92
14. ii.	17·70	8·42	19·79	7·54	19·93	7·48	20·16	7·40
15. ii.	16·62	7·97	13·96	9·48	14·24	9·29	14·09	9·39
Mean	17·86	8·25	17·50	8·49	17·63	8·41	17·79	8·29

With the knowledge that the two widely different methods agreed so closely the high frequency method was worked out and tested. There were two objects: (*a*) to compare all the methods on a dead muscle, and (*b*) to see if there is, or is not, any significant difference between the calibrations of living and dead muscles. For this purpose a series of twelve experiments was made, all reproduced in Table I. Four different measurements were made and sometimes repeated in each experiment,

and the initial heat (I) and the isometric heat coefficient Tl/H (II) of a maximal single twitch were calculated.

Consider first the mean values given in the last row. Here there are only small differences between the different methods of calibration. With calibration of the living muscle the initial heat is slightly greater, and consequently Tl/H slightly less, than with calibration of the dead muscle. Although approaching the limits of error the differences of the averages are probably real; practically, however, the results are equal, but only on the average. In individual cases there are large differences between calibration of the living and calibration of the dead muscles (the different calibrations of the latter being in sufficient agreement). Since these variations, however, are sometimes positive and sometimes negative (from −10·6 to +19·1 p.c.) they almost disappear on taking the average. In order to obtain certain results in a single experiment, or in a small number of experiments, high-frequency calibration of the living muscle is to be preferred, especially if the muscle has been subjected to treatment likely to cause change of shape on killing. The electrocution of the muscle should preferably be done by single induction shocks, since strong tetanization may leave a slight contracture, disappearing only after several hours.

An advantage of the high-frequency method is that in calibration of the living muscle the time expended is much less, because it is not necessary to wait until the muscle is dead and the heat associated with killing has been dissipated. A disadvantage is that it needs more apparatus and experience than the condenser method. The high-frequency method is indispensable if the shape of the muscle is altered in the course of the experiment itself, for example, by the taking up of water owing to osmotic differences. It is necessary in such cases to calibrate the muscle before and after each occasion. Table II gives examples of

TABLE II. High-frequency calibration.

Calibration of	1 mm. deflection = g.cm.	Initial heat, g.cm.	Tl/H	Different experiments
Living muscle	0·1047	18·52	7·98	
	0·1037	18·32	8·06	1½ hours later
Living muscle	0·1421	17·13	8·35	
	0·1417	17·08	8·38	¾ hour later
Dead muscle	0·1072	19·25	9·33	
	0·1058	19·00	9·45	3½ hours later

repeated calibrations on living and dead muscles after different intervals, showing that the method is capable of indicating any significant alteration that may occur in the calibration number.

On the average, and with calibration of the living muscle, the isometric heat coefficient, Tl/H, is about 8·25; the values do not vary considerably from one another, the smallest being 7·52, the largest 9·25. The average initial heat works out at about 0·00348 cal. per g. of living muscle. Although this value is in general agreement with the results of former observers, the "efficiency," as it may be called, the isometric heat coefficient, Tl/H, is definitely larger than that found by Hill [1928] (6·16) and even higher than Blaschko's figures (7–8).[1] Since the method used in tension recording was the same as that employed by these other authors, the results may depend on the condition of the frogs, which varies probably from season to season. It is possible, however, that greater precautions were taken in the present experiments to avoid the possibility of shortening during the supposed isometric contraction (inextensible wire, strong spring, etc.) and to avoid friction at any point in the apparatus.

Whether it would be possible for a muscle to maintain the high value of Tl/H at various temperatures and during continuous activity, as stated by Hartree and Hill [1921] and Hill [1928] for the case of the smaller values found by them, must be left for further work.

Summary.

1. For use on the living muscle a method of high-frequency calibration for myothermic purposes has been worked out and compared with other methods previously used on dead muscles. The comparison on dead muscles gave similar results for (*a*) high frequency, (*b*) audiofrequency, and (*c*) condenser discharges, whereas usually considerable differences, positive or negative, were found in comparison (by high frequency) of the living and dead muscles. Since the calibration constant obtained on the living muscle is sometimes larger and sometimes smaller than that on the same muscle after being killed, there is no important difference between the mean values.

2. Although the heat produced per g. of living muscle in a single twitch in these experiments did not differ from that in former observations (being about 0·0035 cal. per g.), the isometric heat coefficient was found to be larger than generally determined previously. With calibration of the living muscle the absolute value of Tl/H was about 8·25. This value is still rather less than that calculated by Meyerhof from his isometric oxygen coefficient.

[1] Dr Blaschko informs me that a new revision of his protocols gives a mean value of $Tl/H = 8$.

3. If the muscle substance itself is stimulated by applying the cathode of a condenser discharge at the pelvic end of a sartorius, the tension developed is smaller, and the heat produced greater than in stimulation of the tibial part containing the nerve supply (motor point). The reasons for this are discussed.

My sincere thanks are due to Prof. A. V. Hill for his kind hospitality and continual advice. I am very much indebted also to Mr J. L. Parkinson and to Mr A. C. Downing for their valuable help.

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612.627:612.63.031

THE SIGNIFICANCE OF THE LUTEAL ACTION ON THE UTERINE MUSCLE IN THE MAINTENANCE OF GESTATION AND INITIATION OF PARTURITION.

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(*Received March* 19, 1934.)

The development of gestation is accompanied by certain definite alterations in the reactivity of the uterine muscle to oxytocin. In the early stages this posterior pituitary lobe hormone has no effect on the uterus, but later the sensitivity of uterine muscle to oxytocin develops, gradually increases, and reaches a maximum at parturition when very small doses of the hormone are capable of causing contraction. This is true both of the rabbit and of the human subject [Robson, 1933 *a*, *b*]. During the early stages of the puerperium, moreover, the reactivity of the muscle rapidly falls. That a hormone secreted by the corpus luteum plays a part in determining the inhibition of the reactivity of the uterus to oxytocin has been satisfactorily demonstrated both by Knaus [1930] and by Robson and Illingworth [1931], for the injection of certain preparations of the corpus luteum into non-pregnant animals will bring about an inhibitory effect similar to that observed in gestation. Later evidence, however, suggested that the mere withdrawal of the action of the luteal hormone is not sufficient to account for the high reactivity existing at parturition, and it appeared likely that the œstrous hormone might be an important factor in determining the alterations in the physiological state of the uterine muscle during gestation [Robson, 1933 *c*]. The experiments described in this paper were undertaken in order to study further the relation of the luteal secretion to the uterine changes and the factors responsible for controlling the development of uterine activity during the

later stages of pregnancy. They may conveniently be described under two headings, namely:

(1) Changes in uterine reactivity following the cessation of luteal secretion in pseudo-pregnancy, and

(2) Changes in uterine reactivity following the removal during pregnancy of the fœtuses with or without the placentæ.

Methods.

All experiments were performed on mature female rabbits weighing about 2 kg.

The determinations of the *in vitro* reactivity of the uterus were performed as in previous investigations [Robson, 1933 *a*].

A purified preparation of the oxytocic hormone (pitocin) kindly supplied by Parke, Davis and Co. was used.

Results.

Experiments on pseudo-pregnant animals.

Pseudo-pregnant animals were obtained by four methods, namely:

(1) By mating with vasectomized bucks.

(2) By implanting bovine anterior pituitary tissue together with an antiseptic, "quinanil."

(3) By the injection of an alkaline extract from bovine anterior pituitary.

(4) By the intravenous injection of a gonadotropic extract prepared from the urine of pregnant women.

As has been shown previously [Robson, 1932] the procedures described under (2), (3) and (4) result in alterations in the uterine musculature and endometrium similar to those observed in normal pseudo-pregnancy. In all experiments the existence of pseudo-pregnancy was confirmed by laparotomy and observation of the ovaries.

At various stages after the onset of pseudo-pregnancy the animals were operated on and strips of uterus were removed and their reactivity to oxytocin was determined in the usual way, the minimum dose of the hormone necessary to bring about a motor effect being accurately established. The results are collected in Table I. Since pseudo-pregnancy lasts for about 15 days, a number of observations was taken immediately afterwards over a period extending from the 16th to the 19th day. The results thus obtained show that although the cessation of pseudo-gestation is accompanied by a return of the uterine reactivity to oxytocin,

TABLE I. Showing the reactivity to oxytocin of uterine strips removed at various stages after the onset of pseudo-pregnancy.

For explanation of numbers in col. 2, see text.

Animal No. Ra	Methods of induction of pseudo-pregnancy	Reactivity units	Days after onset	Reactivity units	Days after onset	Reactivity units	Days after onset	Reactivity units	Days after onset	Reactivity units	Days after onset
388	(1)	0·5	7	—	—	—	—	—	—	—	—
337	(1)	—	—	0·2	18	—	—	—	—	—	—
366	(1)	<1·0	9	—	—	—	—	—	—	—	—
394	(3)	—	—	0·03	18	0·02	25	0·02	31	—	—
406	(2)	—	—	0·1	16	0·04	20	0·05	28	—	—
407	(2)	—	—	0·02	18	0·02	22	—	—	0·1	35
411	(2)	—	—	—	—	0·05	22	—	—	>0·3	34
412	(2)	—	—	>0·3	19	—	—	0·04	27	0·5	35
420	(4)	—	—	—	—	—	—	0·05	30	0·05	37
421	(4)	—	—	—	—	—	—	0·02	30	0·02	38

the doses of the hormone necessary to cause a motor effect are not particularly small, varying from 0·02 unit to a dose greater than 0·3 unit in a 100 c.c. of solution. Similar observations made at later periods also show that in no case did the muscle react to a dose of oxytocin smaller than 0·02 unit. Experiments were also performed at a later period between the 27th and 31st days after the beginning of pseudo-pregnancy, and thus corresponding to the time when parturition would have occurred had the animals become truly pregnant, but no further increase in the uterine reactivity was observed.

Incidentally it will be seen that the early stages of pseudo-pregnancy are not necessarily always accompanied by a complete loss of reactivity of the uterine muscle to oxytocin; though it must be emphasized that the two cases (Ra 366 and 388) which illustrate this point are exceptional.

The spontaneous rhythmic activity exhibited by the strips *in vitro* was also determined and the results are shown in Table II. In order to provide a basis of comparison with the findings during pseudo-pregnancy, data obtained during that period under exactly similar conditions are included in the table. (These latter experiments were described in a previous communication [Robson, 1932], in which, however, the spontaneous activity of the muscle was not given.) Further, the results obtained in rabbits during pregnancy [see Robson, 1933 *a*] are also given as they will be referred to in the discussion.

The spontaneous rhythmic activity of the uterine strips removed in the early stages of pseudo-pregnancy (3rd–7th days) is, on the whole, rather less than that observed later on (12th–14th day). But the cessation of pseudo-pregnancy (and hence of the specific luteal secretion) is not associated with any increase in the *in vitro* contractions of the uterine

TABLE II. Showing the spontaneous rhythmic activity of uterine strips removed at various stages after the

TABLE II. Showing the spontaneous rhythmic activity of uterine strips removed at various stages after the onset of pregnancy and pseudo-pregnancy.

Animal No. Ra.	Condition	Spontaneous activity	Days after onset	Spontaneous activity	Days after onset	Spontaneous activity	Days after onset	Spontaneous activity	Days after onset	Spontaneous activity	Days after onset	Spontaneous activity	Days after onset
	Pseudo-												
169	pregnant	++	5	.	.	.	.	.	.	.	.	.	.
188	„	+−++	5	++	12	.	.	.	.	.	.	.	.
191	„	++	3	++	14	.	.	.	.	.	.	.	.
197	„	+	3	++	12	.	.	.	.	.	.	.	.
186	„	+	6	++	12	++	16	.	.	.	.	.	.
189	„	.	.	++	11	.	.	.	.	.	.	.	.
190	„	−	6	+	14	.	.	.	.	.	.	.	.
199	„	.	.	+++	12	.	.	.	.	.	.	.	.
203	„	.	.	++	12	.	.	.	.	.	.	.	.
222	„	+	3	++	13	.	.	.	.	.	.	.	.
223	„	.	.	++	12	.	.	.	.	.	.	.	.
368	„	+	7	.	.	.	.	.	.	.	.	.	.
337	„	.	.	.	.	++−++++	18	.	.	.	.	.	.
366	„	.	.	++	9	.	.	++−++	25	+	31	.	.
394	„	.	.	.	.	++	18	++−++	20	++	28	.	.
406	„	.	.	.	.	+	16	+−++	22	.	.	.	.
407	„	.	.	.	.	++	18	++	22	.	.	+	34
411	„	.	.	.	.	.	.	+	22	++++	27	+−++	35
412	„	.	.	.	.	++	19	.	.	++++	30	++++	37
420	„	.	.	.	.	.	.	.	.	++	30	+	38
421	„	.	.	.	.	.	.	.	.	.	.	.	.
334	Pregnant	.	.	.	.	++	18	+++	25	.	.	.	.
336	„	.	.	.	.	++++	18	+	21	.	.	.	.
338	„	.	.	.	.	++	18	.	.	+++	27	.	.
335	„	.	.	.	.	+	19	+	23	.	.	.	.
346	„	.	.	.	.	+	19	.	.	.	.	.	.
342	„	.	.	.	.	.	.	+++	25	+−+++	27	.	.
342	„	.	.	.	.	.	.	.	.	++++	30	++++	32
343	„	.	.	.	.	.	.	+++	25	++++	31	−	.
340	„	.	.	.	.	.	.	.	.	−−++++	28	.	.
389	„	.	.	.	.	.	.	.	.	−	31	+++	38
392	„	.	.	.	.	.	.	.	.	−	32	++	37
339	„	.	.	.	.	.	.	.	.	+−++	33	.	.
371	„	.	.	.	.	.	.	.	.	.	.	++	35
352	„	.	.	.	.	.	.	.	.	.	.	++	39
368	„	.	.	.	.	.	.	.	.	.	.	+++	37
355	„	.	.	.	.	.	.	.	.	.	.	++	37
364	„	.	.	.	.	.	.	.	.	.	.	++	39

muscle which 16–19 days after the beginning of pseudo-gestation are not any more marked than at the earlier stage from the 12th to 14th day. Nor is there any increase at a still later period (22nd–25th days). Very high values for the spontaneous activity were, however, observed in three cases when the strips were removed 27 (one case) and 30 (two cases) days after the induction of pseudo-pregnancy.

Effects of removal of the fœtuses and placentæ.

In eight animals the fœtuses and placentæ were removed at various stages of pregnancy, from the 19th to the 27th day, and the condition of the uterine muscle determined at subsequent periods. The operations were performed with aseptic precautions; small incisions only were made in the uterus and the contents withdrawn; retraction of the muscle reduced the size of the aperture made, so that it could be closed by a single suture. An attempt was also made to remove the fœtuses alone, leaving the placentæ *in situ*, according to the technique first originated by Weymeersch [1912]. When this was performed fairly late in pregnancy, but before the 24th day of gestation, the animals occasionally survived (Ra 395 and 397); similar operations at later stages of pregnancy, however, invariably resulted in the death of the animals, usually within 24 hours. The impression was gained that death had resulted from the absorption of toxic substances from the placentæ which showed signs of degeneration. Mr Hammond very kindly suggested certain precautions which might be taken in the operation, but in spite of these (including strict asepsis) it was impossible to keep the animals alive when the fœtuses only were removed at the later stages of pregnancy.

The reactivity to oxytocin and spontaneous rhythmic contractions of animals in which the uterine contents were removed are given in Table III. An examination of the data shows that in no case was the reactivity to oxytocin particularly high, and that frequently it was very low. Such a result was obtained not only when the interval between the operation and the subsequent experiment was short (24 hours) but also when it was more prolonged. Thus, for example, in Ra 426 uterine strips were removed on the day after the operation and showed a reaction to 0·5 unit of oxytocin. In Ra 402, 2 days after the removal of the uterine contents the muscle was unaffected by 0·5 unit of the oxytocic hormone, and the same dose was just sufficient to cause a contraction of the isolated uterine muscle in animal Ra 349 several days after the removal of the uterine contents.

These results show that the increase in reactivity to oxytocin which occurs during normal pregnancy and culminates in parturition no longer

TABLE III. Showing the reactivity to oxytocin and the spontaneous rhythmic activity (S.A.) of uterine strips, following removal of uterine contents in gestation.

Animal No. Ra	Embryos removed (day of pregnancy)	Reactivity units	S.A.	Day of pregnancy	Interval after removal (days)	Remarks
349	19	0·5	+ + + + − + + +	26	7	—
346	19	0·1	+	23	4	—
415	22	(*a*) > 0·4	+ + + +	24	2	—
		(*b*) > 0·2	+ + + + − + + +	30	8	—
397	23	0·15	+ + + +	30	7	Placentæ left
395	24	0·3	+ + + + − + + +	28	4	,,
405	27	0·2	+ + + +	30	7	—
413	27	0·03	+ + + + − + +	31	4	—
426	20	0·5	+ + + + − + + +	21	1	—
414	26	0·1	+ − + +	30	4	—
402	22	> 0·5	+ − + +	24	2	—

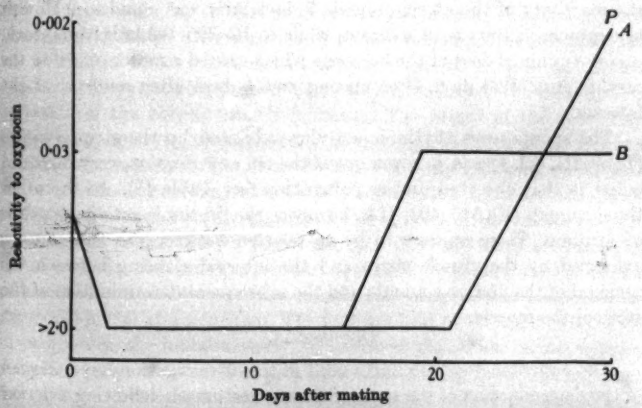

Fig. 1. Schematic representation of the uterine reactivity to oxytocin (expressed as the minimal number of units added to 100 c.c. of Ringer-Locke which causes a motor effect) at various stages of pregnancy and of pseudo-pregnancy. *A*, pregnancy; *B*, pseudo-pregnancy; *P*, parturition.

takes place after removal of the fœtuses and placentæ. This is brought out especially by an examination of the data obtained from strips removed at a time when parturition would have occurred if the uterine contents had not been disturbed. Thus in four cases in which uterine strips were

removed on the 30th day after mating (the uterine contents being removed on the 22nd–27th day of gestation), the dose of oxytocin necessary to cause contraction of the muscle was 0·1 unit or more. In one animal in which the fœtuses and placentæ were removed on the 27th day of gestation, the uterine muscle responded to a concentration of 0·03 unit of oxytocin on the 4th day after the operation.

An attempt was also made to determine whether the presence of the placentæ alone would be sufficient to allow of the development in reactivity observed in normal pregnancy. Two animals, in which the fœtuses alone were removed on the 23rd and 24th day respectively, survived the operation, and uterine strips were subsequently taken out (on the 30th and 28th days after mating), but though the placentæ had not been expelled, the minimum doses of oxytocin necessary to cause contraction of the muscle were still comparatively high. Thus in animal Ra 397 in which the fœtuses were removed on the 23rd day of gestation, the reactivity of the uterine muscle 7 days later was equal to 0·15 unit of oxytocin in 100 c.c. of solution, while in Ra 395, 0·3 unit of oxytocin was the minimal dose of the hormone which caused a motor effect on the uterine muscle 28 days after mating and 4 days after removal of the fœtuses.

The spontaneous rhythmic activity exhibited by the strips *in vitro* (Table III, col. 4) was, in seven out of the ten experiments, very high and equal to that observed during parturition (see Table II). In the other three animals (Ra 346, 402, 414), however, the rhythmic activity was low or medium. There appears to be no relation between the contractions exhibited by the muscle strips and the interval elapsing between the removal of the uterine contents and the subsequent determination of the state of the muscle.

Discussion.

The experimental procedures used in this investigation have allowed of the determination of the state of the uterine muscle following a period of activity of the corpus luteum under two conditions, namely:

(1) Following a period of luteal activity not associated with pregnancy, and

(2) After the cessation of luteal activity occurring under completely normal conditions in a normal though incompleted gestation. In these experiments the pregnancy was interrupted by removal of the uterine contents and this, as Hammond [1917] has clearly shown, results in the degeneration of the corpus luteum of pregnancy and in cessation of its secretory activity.

If the state of the uterine muscle associated with parturition were dependent purely on a withdrawal of the luteal function, then it would be expected that a similar state would be observed when the luteal activity was interrupted under other conditions. Now the state of the uterine muscle at parturition, as far as the reactivity to oxytocin is concerned, can be expressed fairly accurately. In the rabbit the dose of oxytocin necessary to bring about a motor effect on the isolated strips at that stage varies from 0·001 to 0·01 unit in 100 c.c. of solution. It may be added that similar doses are effective, not only on the parturient human uterine muscle [Robson, 1933 *b*] but also on the uterine muscle of the mouse at parturition and at no other stage of gestation. (It is hoped to present details of these findings in the near future.)

An examination of the results obtained in this paper shows definitely that in no case was cessation of luteal activity under any conditions (except normal pregnancy) associated with a rise in the reactivity of the uterine muscle similar to that observed at parturition. Considering first the results obtained after a period of pseudo-pregnancy, it is found that the inhibition of the reaction to oxytocin observed during that period rapidly disappears, this process being dependent upon the cessation of secretion of the corpus luteum hormone. The height of the reactivity attained, is, however, never the same as that seen at parturition. For the purpose of comparison, the data of uterine activity following sterile and non-sterile mating are diagrammatically represented in Fig. 1 which shows that, though after a sterile mating the sensitivity of the uterus to oxytocin begins to increase at about the 15th day, and thus several days before a similar phenomenon is observed in true pregnancy, yet the degree of sensitivity developed by the muscle following pseudo-pregnancy is never as high as at parturition. The findings lead to the conclusion that, in pregnancy, the luteal activity, in as far as the action on the uterine muscle is concerned, is more prolonged than during pseudo-pregnancy, but that nevertheless it does not extend to the end of gestation. This conclusion is also supported by definite evidence that the inhibitory luteal activity during gestation begins to decrease even before the reactivity to oxytocin appears, for vaso-pressin is effective in causing contraction of the muscle in the rabbit shortly after the mid-period of pregnancy [Robson, 1933 *a*] and the inhibitory luteal hormone prevents the action of both the pressor and oxytocic substances on the uterine muscle of the rabbit.

The results following removal of the uterine contents during gestation have led to the same definite conclusion, for the reactivity to oxytocin

of the uterine muscle after such a procedure is in all cases low compared with the sensitivity at parturition. And this result was consistently obtained, even though the uterine contents were removed at different stages during the later part of gestation and the intervals between this removal and the subsequent determination of the state of the uterine muscle varied in the different experiments. These findings thus also lead to the inference that the cessation of the luteal secretion is not in itself sufficient to lead to an exaltation of the uterine reactivity similar to that associated with the physiological expulsion of the uterine contents.

In view of these findings it seems logical to conclude that during these later phases of pregnancy an active mechanism comes into play which brings about the condition of the uterine muscle observed at parturition. And as it has been found that œstrin, in large doses, is capable of causing a rise in the reactivity of the uterine muscle similar to that observed at parturition [Robson, 1933 *c*] it appears quite possible that the mechanism which comes into play during the later stages of pregnancy ultimately, to some extent at least, exerts its effects through the secretion of large amounts of œstrin. The exact nature of the mechanism is unknown, but the finding that it does not become effective when the uterine contents are removed suggests that it may originate in them; and the results obtained in the two cases where the fœtuses alone were removed, the placentæ being left *in situ*, further suggest that the developing embryo is the primary factor which activates this mechanism. It therefore appears justifiable to put forward, as a working hypothesis, the view that during the later stages of gestation the fœtus ultimately controls the development of the reactivity of the uterine muscle to oxytocin and that the secretion of œstrin plays an important part in this effect. The later stages of gestation must also be associated with a cessation of the inhibitory luteal activity, since even large doses of œstrin are unable to overcome the action of the inhibitory luteal hormone [Robson and Illingworth, 1931]; moreover, luteal activity is not essential for the maintenance of the condition of pregnancy in the uterus, as the maximum increase of uterine sensitivity does not result from the mere withdrawal of the luteal secretion. This is in harmony with the finding that, in the human subject, removal of the ovaries does not interfere with the normal course of gestation [Ask-Upmark, 1926].

As regards the spontaneous rhythmic activity of the uterine strips, the evidence derived from a study of pseudo-pregnant animals appears to demonstrate conclusively that cessation of the luteal secretion is not necessarily associated with any definite increase of the contractions

in vitro. And further, there seems to be no appreciable difference between the spontaneous behaviour *in vitro* of strips removed at various stages after a sterile and fertile mating respectively (Table II). On the other hand removal of the uterine contents often, though not always, resulted in the appearance of marked spontaneous contractions, although the reactivity to oxytocin was in these cases conspicuously low. These high spontaneous contractions are not necessarily the result of the cessation of luteal activity and, in fact, the results on the pseudo-pregnant animals suggest that they arise independently of it. In any event it appears unlikely that the rhythmic contractility of the uterine muscle, as exhibited *in vitro*, represents a mechanism involved in the onset of parturition, since, in the human subject at least, it can definitely be asserted that no convincing increase occurs during the course of gestation [Robson, 1933 *b*].

Summary.

The reactivity to oxytocin and spontaneous rhythmic activity of isolated uterine strips were determined in rabbits:

(1) at various stages following the cessation of experimentally induced pseudo-pregnancy, and

(2) at various stages following the removal of the fœtuses with or without the placentæ during the later phases of gestation.

Under these conditions the cessation of luteal activity was not accompanied by an increase in the uterine reactivity similar to that observed at parturition.

The spontaneous rhythmic activity showed no definite increase following the cessation of pseudo-pregnancy, but was usually high in those animals in which the uterine contents had been removed.

The expenses of this investigation were defrayed by grants from the Medical Research Council, the Royal Society, and the Earl of Moray Fund of the University of Edinburgh.

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THE PRODUCTION OF CARDIAC IRREGULARITIES BY EXCITATION OF THE HYPOTHALMIC CENTRES.

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(*Received March* 21, 1934.)

Levy [1912] showed that certain forms of cardiac irregularities could be readily produced in cats under light chloroform anæsthesia by injecting small quantities of adrenaline. He concluded that chloroform rendered the heart "irritable" and that stimulation of the sympathetic produced irregularities in such a heart. Adrenaline, nicotine, barium chloride and other drugs all produced this effect.

Brow, Long and Beattie [1930] showed that in cats decerebration at the Sherrington level abolished the extrasystoles that were induced by light chloroform anæsthesia. They concluded that there was some mechanism above the Sherrington level of decerebration which controlled the cardiac arrhythmias.

As early as 1872 Knoll noted that extrasystolic arrhythmias occurred in experimental animals when the blood-pressure was raised, and it is now generally recognized that cardiac irregularities can be produced in animals either by pressor drugs or by mechanical compression of the aorta.

The fact that disturbances in cardiac rhythm occur as an effect of vagal stimulation has been observed clinically as well as experimentally. Lennox, Graves and Levine [1922] took electrocardiographic records during anæsthesia in man and found that cardiac disturbances were more common in operations on the head and neck than in other operations. The abnormalities were transient, did not cause circulatory disturbance and were of physiological rather than of clinical importance. They concluded that vagal stimulation was in some way responsible for the irregularities.

Rehn [1926] concluded that cardiac disturbances during an operation

were due to nervous impulses from the seat of operation and that the strength of the stimulus to the vegetative nervous system determined the type of irregularity produced.

Hill [1932] investigated the influence of the vagus on extrasystolic irregularities during chloroform anæsthesia in man. Stimulation of the vagus by carotovagal pressure in the neck produced coupled rhythm due to ventricular extrasystoles, and pinching of the exposed nerve produced the same action. These effects were obtained with patients in the third stage of chloroform anæsthesia, who had 1/100 gr. of atropine and in whom no form of vagal stimulation produced slowing of the heart. Hill concluded that increased vagal tone tended to favour the production of extrasystoles under chloroform anæsthesia.

Brow, Long and Beattie [1930] found that stimulation of the central end of the vagus produced cardiac irregularities in cats under light chloroform anæsthesia. They found the same effect after sciatic stimulation and concluded that vagus afferent fibres behaved like other afferent nerves and caused a reflex secretion of adrenaline which was responsible for the irregularity. It is interesting to note in this connection that extirpation of both adrenals did not prevent production of such irregularities.

The author [1934] has shown that both the stimulation of the central end of the vagus and the injection of acetylcholine into the lateral ventricles of cats sometimes caused irregularities in a heart that previously had been perfectly regular in its action. These observations have been repeated and confirmed. The experiments described below suggest that both these procedures produce the cardiac irregularities by acting on certain nerve centres in the mid-brain. These results support Beattie, Brow and Long's view that certain centres in the hypothalamus control the rhythm of the heart.

Experimental methods.

Cats were used for all experiments. The animals were anæsthetized with chloralose (0·08 g. per kg.). Blood-pressure was recorded by a cannula in the right carotid artery and a tracheal cannula was inserted in the trachea for artificial respiration. Both the vagi were dissected and cut in the middle of the neck. A trephine opening was made in the skull at the junction of the coronal and sagittal sutures and intraventricular injections of drugs were made through this hole by inserting a fine needle in the direction of the lateral ventricle. Electrocardiograms (lead II) and kymographic records were taken simultaneously. An injection of 1 mg.

per kg. atropine was given hypodermically about 20 min. before the intraventricular injections were made. Vagal stimulation was effected by stimulating both vagi simultaneously with platinum electrodes.

Effect of vagal stimulation and of intraventricular injections of acetylcholine.

It is well known that stimulation of the central end of the vagus can produce either a fall or a rise in blood-pressure. Irregularities of the heart occur more commonly after central vagal stimulation when there is a rise in blood-pressure than when there is a fall. The irregularities may occur during or after cessation of the stimulus. They persist for a short time only and after this the heart becomes regular again. Injections of small quantities of acetylcholine into the lateral or third ventricle of cats also precipitate irregularities in a rhythmically beating heart. Such injections can produce either a rise or a fall in blood-pressure, and in this case also the irregularities occur more frequently when there is a rise in blood-pressure than when there is a fall, but they may also occur when the blood-pressure is not much affected by the injection. These irregularities also are transient and the heart regains its regular rhythm in a short time. Fig. 1 shows firstly the effect of stimulation of the central ends of the cut vagi. This caused a temporary arrest of respiration and a rise of blood-pressure which was followed by cardiac irregularities. An injection of 0·4γ of acetylcholine did not produce any effect on respiration but caused cardiac irregularity. A second injection of 0·8γ of acetylcholine caused respiratory arrest and more pronounced cardiac irregularity. The figure shows the striking similarity between the effects of central vagal stimulation and intraventricular injections of acetylcholine. Moreover it shows that cardiac irregularities can be produced without changes in either respiration or blood-pressure. The author [1934] has advanced the provisional hypothesis that stimulation of the afferent fibres of the vagus causes liberation of acetylcholine in the central nervous system, and that this may be the reason for the similarity in effects seen in Fig. 1.

Brow, Long and Beattie [1930] concluded that the brain centre causing cardiac irregularities was situated above the Sherrington level, and the author's results support this conclusion, for he found that after decerebration below the Sherrington level application of acetylcholine to the brain even in as high concentrations as 1 in 100,000 did not produce any action on the rhythm of the heart.

Irregularities of the heart can also be produced by various other means. Injections of caffeine citrate (0·25–0·5 c.c. of 5 p.c. solution) in the lateral ventricle of cats produced a marked rise in blood-pressure and a marked cardiac arrhythmia which persisted for a fairly long time, and small doses of nicotine also had the same action. The arrhythmias can also be produced by clamping the common carotid arteries in the neck or by injecting air into the carotid artery.

Fig. 1. In this and subsequent tracings *R* = respiration, B.P. = blood-pressure. The scale on the left measures B.P. in mm. Hg. Cat (wt. 2·3 kg.) anæsthetized with urethane. A, stimulation of the central end of both vagi. B, injection of 0·4γ of acetylcholine in the right lateral ventricle. C, injection of 0·8γ of acetylcholine in the right lateral ventricle. Time = 10 sec.

Influence of changes in blood-pressure on cardiac irregularities.

All the procedures mentioned above, namely intraventricular injections of acetylcholine, caffeine or nicotine and clamping of the carotids, usually caused a marked rise in blood-pressure, and the question arises whether this may not be the cause of the cardiac irregularities. Levy [1913] pointed out that there was a close relation between the blood-pressure level and occurrence of ectopic beats. In all forms of stimuli used by Levy there was a rise in blood-pressure. Hering [1900]

showed that marked resistance to the action of the heart brought about by compression of the aorta near its arch gave rise to extrasystolic arrhythmia. Beattie, Brow and Long [1930] pointed out that the stimulation of the sympathetic system brought about during the operative procedure of clamping the aorta and the asphyxiation of the central nervous system which such an operation necessarily involved, were possible causes of these effects. Karplus and Kreidl [1911, 1927] showed that there was a well-localized region in the hypothalamus, stimulation of which led to a well-marked rise in blood-pressure. Since any pressor action can bring about extrasystolic arrhythmia it becomes doubtful whether all the procedures discussed above have an action on a special centre in the brain governing the heart or are the result of the rise in blood-pressure produced by their action on the centre of Karplus and Kreidl. It has indeed been shown that intraventricular injections of acetylcholine can produce cardiac arrhythmias when there is no appreciable change in the blood-pressure level (cf. Fig. 1), but intraventricular injections of caffeine and clamping of the carotids always cause a rise of blood-pressure and hence the mechanism by which they produce cardiac irregularities is doubtful. Further evidence on this point was obtained by studying the effects produced by hypnotics of the barbituric acid series which are believed to exert a selective depressant action on the mid-brain. Intraventricular injections of caffeine and clamping of the carotids were selected as the best agents for provoking cardiac irregularities, because their effects are more marked and more persistent than the other agents with a similar action.

Effect of sodium barbitone on cardiac irregularities.

The action of sodium barbitone in inhibiting the cardiac irregularities produced by agents mentioned above was studied. The sodium barbitone was either applied locally to the hypothalamus or else was injected intravenously. Fig. 2 shows the irregularity of the heart produced in a decerebrate animal with chloroform and adrenaline. The level of decerebration was well above the Sherrington level in this experiment. The level of blood-pressure was not high and a control injection of 0·05 mg. of adrenaline before administration of chloroform produced a good rise in blood-pressure but no cardiac irregularity. Chloroform by inhalation and 0·05 mg. adrenaline by intravenous injection produced the typical arrhythmia shown at the commencement of Fig. 2. A 5 p.c. solution of sodium barbitone (0·3 c.c.) was then applied directly to the hypothalamic nuclei. The effect is seen in the figure. There was an immediate action on

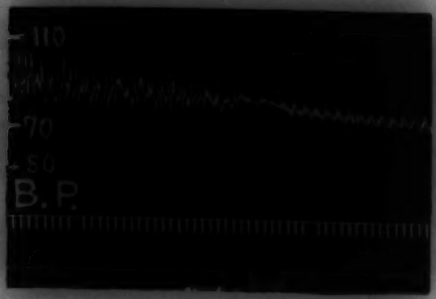

Fig. 2. Decerebrate cat (wt. 3·1 kg.), blood-pressure showing irregularity of the heart provoked by previous administration of chloroform and adrenaline. At the signal 0·3 c.c. of 5 p.c. solution of sodium barbitone applied to the hypothalamus. Time 2 sec.

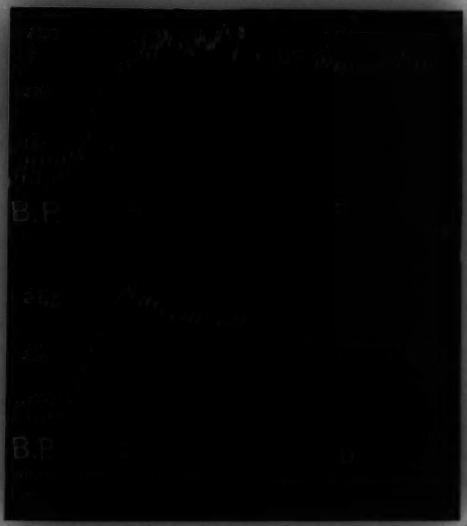

Fig. 3. Cat (wt. 1·5 kg.), anæsthetized chloralose. Blood-pressure. A, intraventricular injection of 15 mg. of caffeine citrate at the signal. B, tracing 5 min. after A. C, intraventricular injection of 15 mg. of caffeine citrate at the signal after an intraventricular injection of 0·025 g. sodium barbitone. D, tracing 5 min. after C. Time 2 sec.

the rhythm of the heart although there was no marked change in the level of blood-pressure. It is interesting to note in this connection that the effect of sodium barbitone lasted for a short time only (about 20 min.), and after this interval administration of chloroform and adrenaline again produced an irregular heart, and this irregularity was abolished by a second application of sodium barbitone.

Fig. 3 A shows the irregularity of the heart after an intraventricular injection of caffeine citrate (5 p.c. solution, 0·3 c.c.) in a chloralosed, atropinized cat. The injection was given through a small hole trephined

Fig. 4. Electrocardiograms taken during experiment shown in Fig. 3. A, taken during period B (Fig. 3). B, taken during period D (Fig. 3). Time 1/5 sec.

at the junction of the coronal and sagittal sutures. There was a rise in blood-pressure and some irregularities of the heart immediately after the injection. The irregularities became most marked about 5 min. after the injection (Fig. 3 B) and the heart remained irregular for about 20 min. The electrocardiogram taken after 5 min. shows the character of the irregularities (Fig. 4 A). An intraventricular injection of sodium barbitone (0·25 c.c. of a 10 p.c. solution) was given when the heart was showing a few irregularities. This injection produced a slight rise in blood-pressure and slightly increased the irregular action of the heart for a short time. The heart however regained its regular rhythm immediately afterwards and remained so for about 15 min. A second injection of caffeine (0·3 c.c. of a 5 p.c. solution) was then given in the same way as the first one. This

produced a rise in blood-pressure, almost about the same as the first one but very few irregularities of the heart (Fig. 3 C). The tracing to the right (Fig. 3 D) shows the kymographic record taken 5 min. after the injection and it will be seen that the heart is quite regular. An electrocardiogram taken simultaneously also shows the regularity of the heart (Fig. 4 B). The difference between the action of caffeine given intraventricularly before and after intraventricular injections of sodium barbitone is well seen both from the kymographic and electrocardiographic records. As

Fig. 5. Cat (wt. 3·2 kg.), anæsthetized chloralose. Drum turned back to get superimposed tracings. Lower tracing taken before and upper tracing after intravenous administration of 0·2 g. sodium barbitone. Carotid arteries clamped at the arrows. Time 2 sec.

the rise in blood-pressure produced by the two injections of caffeine was about the same, it appears that the pressor effect is not the cause of the irregularities.

Clamping of the carotids is the simplest method of producing a rise in blood-pressure without introduction of a drug. The rise is usually accompanied with irregular action of the heart. The lower tracing of Fig. 5 shows that a rise of blood-pressure produced in this way was accompanied by extrasystoles. After this tracing had been obtained the cat was given 0·2 g. of sodium barbitone intravenously, and after this clamping of the carotids produced a rise of blood-pressure without any extra-

systoles (upper curve, Fig. 5). As clamping of the carotids after sodium barbitone produced about the same rise in blood-pressure, it is evident that the rise itself is not responsible for the irregularity. This experiment further indicates that the reflex initiated by a fall in the carotid sinus pressure passes through two mechanisms—vaso-pressor and cardiac regulator and that sodium barbitone depresses the latter but not the former.

Influence of sodium barbitone on ectopic beats in man.

The effect of sodium barbitone on the incidence of ectopic beats was studied in a normal, healthy, young subject showing ectopic beats.

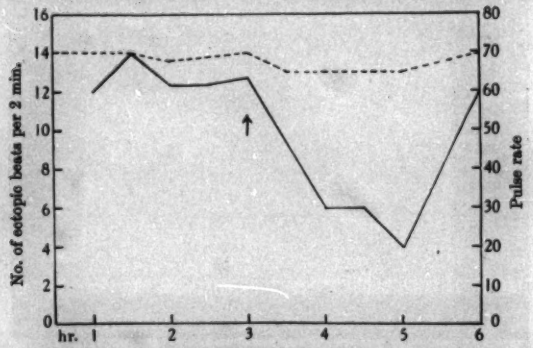

Fig. 6. Effect of 1 g. sodium barbitone (taken by mouth at arrow) on incidence of ectopic beats per 2 min. (continuous line). Dotted line (pulse rate).

Irregularities were counted by feeling the pulse at the wrist and electrocardiograms were taken every half hour. Sodium barbitone (1·0 g.) was given orally. It was found that the number of ectopic beats increased slightly within the first 10 min. of administration of the drug and dropped to less than half within the next 30 min. This effect was maintained for about 2 hours and the irregularities returned within 2½ hours. Fig. 6 shows the effect of 1·0 g. of sodium barbitone on the pulse rate and incidence of ectopic beats. The rate of pulse slowed to a slight extent but there was a marked effect on the number of ectopic beats. The action however passed off within 2½ hours, rather abruptly.

Larger doses of sodium barbitone have a more prolonged action. The

effects of large doses (2·0 g.) of the drug were studied in the same subject. The number of extrasystoles was counted by feeling the pulse at the wrist. The normal variation in the incidence of ectopic beats was observed for a week, and it was found that the number of extrasystoles gradually increased during the evening and was maximum at about 11 o'clock at night. This increase in the incidence of ectopic beats persisted for about 2 hours. Sodium barbitone was therefore given at 10 o'clock at night, and it was found that the number of ectopic beats was markedly reduced by the drug and the effect lasted for more than 3 hours. The results are shown in Fig. 7.

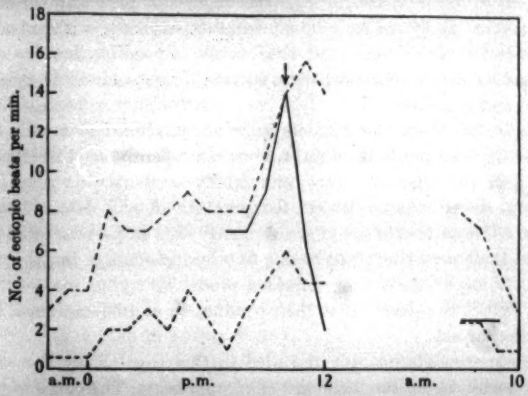

Fig. 7. Effect of 2 g. sodium barbitone (taken by mouth at arrow) on incidence of ectopic beats per minute (continuous line) in subject B.B.D. Dotted line, highest and lowest figures obtained during 7 days' control observations.

Discussion.

There are two theories advanced to explain the occurrence of ectopic beats. The theory of re-entry is based on the experimental observations of Mines [1913] that certain muscle fibres are in a refractory phase and do not respond to the stimulus but become responsive as the impulse travels along the other fibres and comes back to the point of stimulus. The theory of parasystole explains ectopic beats by assuming the existence of a focus in the heart which is capable of sending out but not of receiving impulses. Both these theories are myogenic, and this theory is supported by the fact that digitalis in large doses regularly produces extrasystoles and this drug almost certainly acts directly on the myo-

cardium. Hence the action of caffeine in producing extrasystoles has generally been ascribed to the direct action of the drug on the myocardium. In fact the neurogenic view has found little favour with physiologists and clinicians alike.

The experiments of Brow, Long and Beattie have proved the existence of a centre in the hypothalamus, stimulation of which can cause cardiac extrasystoles. Experiments described in this paper support their view. The fact that all experimental procedures which produced irregularities were effective after bilateral vagal section and atropinization of the animal proves that the efferent impulses which provoke cardiac irregularities do not pass through the vagus but probably through the sympathetic. It is further evident from experiments with intraventricular injections of drugs that the centre responsible for the cardiac irregularities can be stimulated by various drugs such as acetylcholine, caffeine and nicotine.

The ectopic beats met with clinically are produced by a wide variety of causes such as drugs, bacterial toxins, reflexes produced by disordered function of the digestive tract, and finally they may be produced by emotional disturbances without the presence of any definite lesion or disorder. Ectopic beats are very frequently seen in persons of advanced age, and these are believed to be due to sclerotic changes in the coronary vessels. Ectopic beats are therefore produced by so many different factors that it is unlikely that their production can be explained by one single mechanism.

The experiments on man recorded in this paper show the effect of sodium barbitone on the incidence of ectopic beats. The cause of ectopic beats was unknown in this case, but a central nervous depressant like sodium barbitone markedly lessened the number of ectopic beats. Extrasystoles produced experimentally in cats by central drug action were also abolished by administration of sodium barbitone. Therefore probably the extrasystoles in the case referred to above were of central origin.

Beattie, Brow and Long [1930] produced extrasystoles in cats by injecting caffeine intravenously in an amount equal to 35–45 mg. per kg. of body weight. They observed that caffeine produced extrasystoles in a decerebrate animal, after this had been without an anæsthetic for 5 hours, and that section of all nerve connections to the heart and removal of the suprarenal glands had no effect whatever on the irregularities of the heart produced by intravenous injections of caffeine. They therefore concluded that the site of action of caffeine was either on the myocardium or the sympathetic nerve endings in the heart.

I injected caffeine intravenously in cats and found that the dose required to produce extrasystolic arrhythmia was about 50 mg. per kg. of body weight. Intraventricular injections of caffeine in an amount equal to 10–12 mg. per kg. were however sufficient to produce the cardiac irregularity. The fact that the minimum effective intraventricular dose of caffeine was one-fifth of corresponding intravenous dose is strong evidence against the drug acting on the heart and indeed against its acting on any tissue outside the central nervous system.

The power of caffeine to produce extrasystoles in animals is of clinical interest because caffeine poisoning is a known cause of extrasystoles in man. The dose of caffeine given intravenously in the animal experiments is equivalent to a dose of 3·0 g. in a human being, whereas the daily consumption of caffeine by a heavy tea drinker is likely to be about 1 g. Sollmann [1932] states that doses about 1 g. of caffeine may produce alarming symptoms.

In comparing the effects on animals to human beings it must be remembered that the animals were anæsthetized and that immoderate tea drinking only produces cardiac irregularity in a certain proportion of susceptible cases. The difference in animal and human dosage is therefore not so great as to preclude the possibility of a similar mode of action in the two cases. It has already been shown that in animals the production of extrasystoles by caffeine is probably due to some actions on the central nervous system. It is therefore possible that caffeine can produce extrasystoles in man in a similar way.

Summary.

1. Central vagal stimulation and injection of small quantities (0·4–1γ) of acetylcholine into the ventricles of the brain produce cardiac irregularities in cats.

2. Similar irregularities can be produced by intraventricular injections of caffeine and nicotine or by clamping both the carotid arteries.

3. Sodium barbitone given intracerebrally or intravenously lessens or abolishes cardiac irregularities provoked by caffeine or by clamping of the carotid arteries.

4. Large doses of sodium barbitone lessen the incidence of ectopic beats in man.

5. The evidence indicates that caffeine can produce cardiac irregularities by some action on the hypothalamic centres.

My thanks are due to Prof. A. J. Clark for his suggestions and help. The expenses of this research were defrayed by a grant from the Moray Fund of Edinburgh University.

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THE "WEVER AND BRAY PHENOMENON." A STUDY OF THE ELECTRICAL RESPONSE IN THE COCHLEA WITH ESPECIAL REFERENCE TO ITS ORIGIN.

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(*Received March* 29, 1934.)

In 1930, as is now well known, Wever and Bray [1930 *a*, *b*] first described certain electrical phenomena occurring in the trunk of the acoustic nerve during the reception of auditory stimuli. This effect, which has since come to be known as the Wever and Bray phenomenon, consists essentially of potentials alternating at a frequency accurately corresponding to that of the stimulating sound. Thus, with suitable amplification, speech is clearly reproduced and the voice of the speaker recognized. After excluding certain artefacts, Wever and Bray satisfied themselves that the effect was a vital one, in that it was dependent upon the functional integrity of the cochlea; and further, concluded that it was attributable to true action potentials produced in the fibres of the auditory nerve. They mention the possibility of its being the expression of diffuse electrical changes, engendered in the cochlea, in a manner not suggested, but prefer the first explanation for the following reasons:

(1) That the phenomenon was more marked with the active electrode in the brain stem than in any other position equidistant from the cochlea, thus following the lines of nervous conduction.

(2) That with the active electrode in the brain stem, and sound passing into one ear (the opposite cochlea having been destroyed) the effect was abolished by cutting the auditory nerve of the stimulated ear, although the gap between the cut ends of the nerve was filled with a conducting medium, *e.g.* saline solution. With the electrode on the distal stump of the nerve, the effect was still demonstrable.

[1] Working with a grant from the Medical Research Council.

In 1931 Adrian [1931 *a*], on the basis of his own repetition of, and further experimental findings on this work, concluded against the neural theory of its origin, the two reasons adduced being:

(1) The very wide distribution of the electrical disturbance, it being possible to pick up the potential changes from almost any point around the cochlea, apart from (but of course, including) the nerve.

(2) With the electrode on the nerve, conversion of the nerve distal to the electrode into an inert conductor, by freezing or the injection of novocain, failed to interfere with the potential changes picked up.

Hence, in contradiction to the second of Wever and Bray's experiments described, the nerve trunk appeared to behave as an inert conductor, in transmitting electrical oscillations of possibly non-neural origin. Adrian remarks [1931 *b*] that Wever and Bray's nerve section experiment "is certainly in favour of the nerve impulse explanation," but points out that the abolition of the response may be attributable to the concomitant severing of the vascular supply to the internal ear. Despite this conflicting evidence as to their origin, it has since become generally agreed that the presence of alternating potentials in the eighth nerve is chiefly due to the spread of electrical changes engendered within the cochlea, in a manner at present unknown, the nerve here acting as an inert conductor.

It is towards the solution of the problem of the origin of the cochlear effect that the experimental work described in this paper has been directed.

Apparatus, and operative methods.

A schematic diagram of the apparatus is given in Fig. 1. The source of sound used throughout these experiments has been a heterodyne oscillator, coupled through an amplifier to a loud-speaker. The oscillator used provides approximately 9 volts, R.M.S. A.C., at frequencies from 50, or lower, to 12,000 cycles. Between 50 and 12,000 cycles the output contains not more than 1 p.c.[1] harmonic, expressed as a percentage of the total output. This is fed into an attenuator, as shown; this latter gives an overall attenuation of 60 decibels, in steps of 2 decibels, and is accurate to within some 2 p.c.[1] between the limits of frequency indicated. The attenuator is separated from the loud-speaker by a triode output stage, which is transformer-coupled to the loud-speaker. This is enclosed in a sound-proofed box, as it is found that with a diffused sound, as from an unenclosed speaker, the pressure increases and decreases simultaneously

[1] These figures are those given by the respective makers; we have had no cause to doubt their accuracy, but have not ourselves made check measurements.

on both sides of the tympanic membrane of the preparation, the middle ear of which is exposed to the air. This leads to a "cancelling out" effect, strikingly displayed in some preparations by a marked falling off in amplitude of response, upon removal of the bony septum separating the cavity of the bulla from the middle ear. In addition, considerable freedom from microphonic pick-up in the amplifier is secured, an additional difficulty with diffused sound. The sound is led to the ear of the preparation by rubber tubes, wrapped with "slag" wool and cloth; this is effective

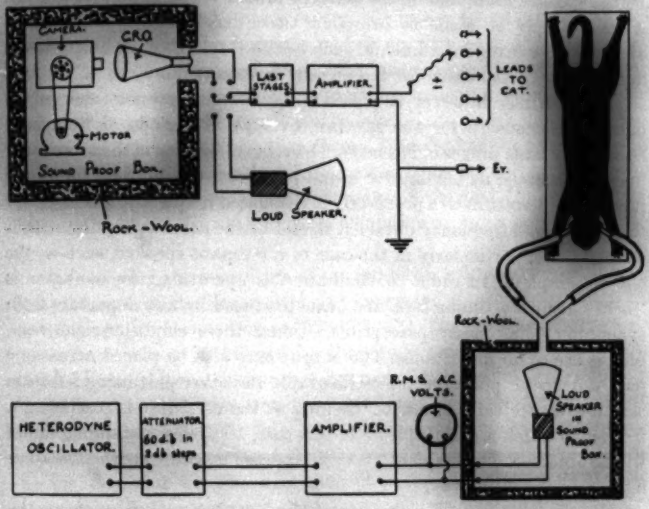

Fig. 1.

in preventing tones of high frequency, and therefore short wave-length, from reaching the preparation or the amplifier, from this source. The tubing connects with a small brass tube, inserted in the meatus of the preparation; this is normally tied securely in the meatus, and thus some freedom is obtained from intensity variations due to movements of the preparation or the source. No reliance may be placed upon day to day comparisons, however, as the wave-length, and thus (owing to the properties of the tubes) the intensity, of sound of a given frequency varies with temperature, humidity, and pressure; in addition, the elastic and other properties of the tubes are by no means constant, and thus the

absolute intensity for a given loud-speaker current may vary by quite large amounts.

In addition, the loud-speaker may not be relied upon to give constant intensities for constant energizing currents, at given frequencies, as the frequencies to which it is resonant may change quite considerably, with atmospheric and temperature fluctuations; it is clear, therefore, that the intensity of sound at the tympanic membrane of the preparation may fluctuate within quite large limits, in respect of variable factors operative on the speaker itself, and on the delivery tubes over large periods of time. Attempts may be made to overcome these deficiencies, by the use of absolute measuring instruments, such as the Rayleigh disc, but this was not considered necessary for the present investigation.

Reliance may, however, be placed upon the sound-pressure energizing-current relationship for the speaker, over short periods of time, and reasonably large ranges of intensity. Thus the calibration of the attenuator may be accepted as holding for sound-pressures, within these limits. If it is desired to return to a previously investigated frequency, it is essential that the second frequency obtained should be the same as the first within a small limit, particularly in the case of a resonant speaker, such as the one we employ. In order to facilitate this operation, the oscillator is "held" against a tuning fork, the beats produced by any departure from synchrony being easily perceptible. Under these conditions the resonances are of no importance. The output may also be placed across one pair of deflector plates on the oscillograph, the other pair being fed from the time-controlled A.C. mains. So long as the oscillator is producing a frequency which is a multiple of, in this case, 50 cycles, a standing figure will be obtained. The frequency may be assessed by counting the Lissajous figures, in the usual way.

For observation and recording of the impulses picked up from the preparation, an amplifier of high gain, and a cathode ray oscillograph are used. The amplifier is constructed straight-forwardly, triodes being coupled successively by resistances and condensers. The use of high values of coupling condenser and gridleak, together with relatively low values of anode resistance, ensures fair frequency linearity over a large range. Tests with the heterodyne oscillator referred to above showed that the gain of the amplifier was constant to within 10 p.c. between 50 and 3000 cycles, above which frequency the magnification fell off, being approximately 6 decibels down at 5000 cycles. Calibration at all frequencies employed is carried out during the experiments enabling the voltages developed by the preparation to be assessed within small limits. A gain

control, consisting of a grid-potentiometer, is placed between the second and third stages, and between the fourth and last stages. By proper adjustment of these, linear distortion may be avoided, an important point, in view of the work on wave-form, to which reference is made elsewhere.

For much of the work, particularly at high frequencies, a push-pull input stage was used[1] [Matthews, 1934]; this gave considerable freedom from electrostatic and electromagnetic pick-up, both of interference and of the voltages feeding the stimulating loud-speaker. This latter was, of course, especially important at high frequencies, and it was found that with a plain input stage, using control electrodes on an indifferent part of the preparation, the voltages picked up were such as to be clearly audible in the loud-speaker, when high magnifications were employed. This phenomenon was reduced almost to vanishing point, when the push-pull stage of similar gain was in use.

The oscillograph tube used is a Cossor Type C which we find extremely satisfactory. The actinic value of the spot or figure is high; all the records obtained have been secured with 1000 volts on the gun. Under these conditions, the life of the tubes is some 500 hours or more, with reasonable care.

For studying the wave-form of the impulses, a time-base unit is used. This, which is of the linear type, is constructed in accordance with the circuit given by Messrs Cossor [1933, p. 23]. The essential features are that a linear sweep is obtained by charging a condenser through a constant current device, in this case, a screened high-frequency pentode, so biased as to be operative over the "flat" part of its anode-volts anode-current characteristic; the "fly-back" discharge is obtained by means of two hard valves, so connected that the second has a cumulative effect on the first. In this way, an arrangement is secured which possesses advantages over the saturated-diode-gas-discharge-tube sweep circuit, in that it is much more nearly linear, and is practically independent of fluctuations in supply voltage; we are thus enabled to use the rectified and smoothed A.C. mains for energizing purposes. In addition to the "saw-tooth" and "work" voltages, arrangements are made whereby a continuously variable D.C. voltage may be applied across either pair of deflector plates in the tube; thus it is possible to "shift" the image about the screen of the tube, or move it against a marked line. If the negative and positive peaks of the incoming "work" voltage are successively shifted against the line, in this way, the peak voltage across the work

[1] For this suggestion, we are much indebted to Mr B. H. C. Matthews, of the Physiological Laboratory, Cambridge.

plates may be read off; this is done by subtracting the smaller from the larger D.C. voltage required to move the image thus; the measurements may conveniently be made by means of a D.C. meter, connected between one deflector plate and gun.

As can be seen from the diagram, the output from the amplifier may be switched over to a loud-speaker; in this way it is possible to detect much smaller audiofrequency voltages than are visible on the oscillograph, as on the latter, these may often be "masked" by other voltages, produced by small movements of the preparation, injury potentials, and the like, or by amplifier disturbances (valve hiss, etc.), which may be considerable at high gains. In the loud-speaker the former are easily heard as "whistles," superimposed on a background of "s-sh" noises.

Photographic records are obtained by means of a moving film camera, using 35 mm. cine bromide paper moving at speeds from 9 in. up to 10 ft. per second. Owing to the necessity of preventing the noise of the camera reaching the preparation, it was found essential to enclose the camera and face of the oscillograph in a sound-proofed box constructed of fibre compound and slag wool. For photography of course, the sweeping voltages referred to above are cut out, leaving the spot operated in the horizontal plane by the work voltages alone.

A change over from inspection to photography can be effected without serious loss of time, by the operation of two switches and the closure of the lid of the sound-proofed box.

Points, which need mention, in the operative technique employed are:

(1) *Anæsthesia.* Decerebration was carried out in many of the preparations under ether. In others, light nembutal anæsthesia was found to be the method productive of best results.

(2) *Immobilization of the preparation and electrodes.* The cat's head was securely immobilized by a large clamp operating upon the zygomatic eminences, the handle of this clamp being fixed through a universal joint to a steel pillar rigidly seated on the table. By this means, any position of the animal's head could be obtained, and maintained with satisfactory rigidity. The electrodes were held in holders incorporated in the jaws of the clamp described, thus rendering it possible to move preparation and electrodes *en bloc.*

The electrodes employed were generally of platinum, the silver silver-chloride type also being used on occasions. All work upon the preparation itself was carried out in a cage of galvanized iron wire, carefully earthed.

Hence screening of the leads from the preparation within this cage was not found to be necessary, particularly when the push-pull input stage described was in use. Suitable screening of the short length of lead between the amplifier and cage was employed.

(3) *Introduction of the stimulating sound into the cat's ear.* Insertion of the end of the sound tube into the external ear we have found a quite unsuitable method, owing to the extreme convolution of the outer parts of the passage of the external ear. The end of the tube either impinges upon the meatal wall, or at other times lies free in the lumen, with resulting variations in the intensity of the stimulus. In addition, no means is provided for an inspection of the drum-head and meatus itself; this is necessary, as a considerable number of animals employed (some 40 p.c.) are found to have the depths of the meatus packed with cerumen. The responses obtained from these are, for obvious reasons, atypical, and worthless for experimental purposes. We have proceeded as follows: Dissecting from the dorsal surface, the external meatus is exposed, and divided approximately 1 cm. from the membrane. By the use of a frontal mirror the depths of the meatus and the tympanic membrane can be inspected. A brass tube is then securely tied into the stump of the meatus, to this the sound tube being attached in the manner previously described.

Experimental results.

Working with the apparatus and technical methods described, the following attributes of the cochlear effect have been investigated.

(1) We have succeeded in confirming Adrian's suggestion that the abolition of the response in the auditory nerve consequent upon its section [Wever and Bray, 1930 *a*] is due to concomitant interruption of the vascular supply of the internal ear. It is possible in favourable preparations to divide the nerve without significant injury to the internal auditory artery, this lying in a well-defined connective tissue strand, anterior to the nerve. If this is done, the response from the central end of the cut nerve persists, but disappears promptly when the vascular strand is divided.

(2) *The relative amplitude of the voltages developed between various points on the cochlea, with reference to a diffuse earthed electrode in the neck muscles.* Adrian [1931 *a*] was the first to emphasize the general nature of the amplitude distribution of the phenomenon about the temporal bone, also to indicate the relatively high amplitude of the impulses picked up from the fenestra rotunda. Davis and Saul [Davis, Derbyshire,

Lurie and Saul, 1934] have confirmed this, noting too, the subarcuate fossa, as giving a good response. Adrian [1931 *b*] suggests that the explanation of the large size of the voltages developed between the diffuse electrode and the round window is that there here exists a path of low resistance, a large percentage of the voltage developed within the cochlea being, therefore, effectively across these points. The voltages picked up from the surface of the bone are lower, owing to the resistance of the bony capsule. The electrical distribution is, however, difficult to envisage. It is possible to take the view that the large size of the voltages at the round window is due not merely to the existence here of a path of lowered resistance, but to the fact that the voltages developed within the cochlea here have a higher value than those developed elsewhere. This question is of theoretical importance for this reason; on the Helmholtz theory it is supposed that sounds of low frequency engender stimulation of sensory cells or nerve endings confined to the apical region of the cochlea; it would be reasonable to suppose, therefore, that on Wever and Bray's "Volley-resonance" hypothesis the electrical response for low tones would tend to be maximal towards the apex.

With a positive electrode upon the surface of the cochlea it has been found that the response exhibits a parallel fall in amplitude for high and low frequencies (250 to 4000 ~) as the electrode is moved from the round window towards the apex. This would accord with the view that the bony capsule constitutes an efficient insulating medium; all potential changes generated within the cochlea having a maximum value at the round window and following thence a path of resistance equal for high and low frequencies along the mucous membrane to an electrode placed upon the surface of the cochlea.

In order to eliminate the factor of resistance of the bony capsule which tends to make the response for all frequencies maximal towards the fenestra rotunda the following technique was adopted:

After reflection of the mucous membrane two small holes were drilled in the cochlear wall with a dental drill, one at the base close to the attachment of the round window membrane, the other towards the apex, the operation being carried out under inspection through a dissecting microscope. The bone is dense and avascular, and may be some millimetres in thickness, characteristics well in accordance with the view of its good insulating properties. In this way small concavities were formed with bony floors the thickness of which could with practice be reduced to a very small order indeed, this state being manifested by a wetness recognizable through the microscope and due to the passage outwards of a minute

quantity of the cochlear fluid. When thus completed the concavities were filled with small mercury beads delivered from a micro-pipette, these acting as seals to prevent the further escape of fluid and furthermore establishing good electrical contact locally with the interior of the cochlea.

The amplitude of the response obtained when leading off from a platinum electrode making contact with either of the mercury beads was found to be considerably augmented as compared with that obtained from the intact cochlear wall. The response from the round window itself was found to be almost unaltered, a point of importance in showing the absence of significant damage to the cochlea by the drilling operation described.

The relation between the response from the apical and basal mercury electrodes is shown in the following table:

Frequency of stimulus (cycles/second)	Response at apical electrode microvolts	Response at basal electrode microvolts
250	150	45
2050	30	110

It is worthy of note that, as previously recorded [Hallpike and Rawdon-Smith, 1934], the response at the apical electrode shows a progressive rise in amplitude, as the frequency falls, whilst that at the basal electrode shows a corresponding fall. These results have been repeated without significant disparity on 4 successive cats.

Two theoretical considerations arise from these experimental data. Firstly, they are fully in accordance with the view that the bony cochlear wall constitutes an efficient insulator of the potentials generated within; further, the good response normally obtained from the acoustic nerve and the fenestra rotunda is adequately explicable as being due to the existence at these points of paths of low electrical resistance.

Secondly, the hypothesis that the vibrating elements of the cochlea are differentially tuned for frequency is considerably strengthened. It must be stressed, however, that no indication as to the mode in which frequency is discriminated psychologically is given.

The origin of the cochlear effect is not clearly indicated by this experiment. It is likely that the response would be maximal at the region of maximal mechanical disturbance, whether its origin was non-neural or neural. Further consideration of this point is given below.

(3) Studies of the wave-form of the cochlear effect, employing the cathode ray oscillograph in conjunction with the linear time sweep device described, have revealed that, within the frequency range 500 cycles to

1000 cycles, and employing tones of intensity some 20 decibels above the human threshold, the response obtained is sometimes remarkable in that the waves show not only a quite close approximation to true sinusoidal form, but also a high degree of serial uniformity, not only for form but also for amplitude (*vide* Fig. 2). This response may persist unchanged with continued sound stimulation for as long as half an hour.

Adrian [1932] reminds us of the possibility of obtaining from groups of touch receptors, reproduction of a stimulation frequency up to 400

Fig. 2. Wave-form of the cochlear effect. Synchronized linear sweep used, plate exposed for one-fifth second, giving 200 superimposed waves. Frequency of stimulus, 1000 cycles. (Untouched photograph.)

cycles or so, the wave-form of the synthesized response being very approximately sinusoidal. Wever and Bray's hypothesis of the origin of the cochlear phenomenon is, in effect, an extension of this mechanism. We would indicate the difficulty of accounting for the synthesis of waves of these characteristics on such a theory.

(4) Adrian [Adrian, Bronk and Phillips, 1932] states that cocaine injected *via* the round window reduces or abolishes the cochlear effect, and considers this a point which favours the neural hypothesis of its origin. As Adrian stated, the introduction of cocaine through the round window

membrane by a needle is a method uncertain in the possibilities of excluding other forms of damage to the cochlea. We have preferred, therefore, the method of applying cocaine in the form of crystals to the membrane. The results of this operation are a rapid and steady decrease in the response, and its entire disappearance at the end of about 5 min. That this may be interpreted on the non-neural hypothesis as a disturbance of the intracochlear mechanics, or of possible polarized structures (by, for example, osmotic pressure changes), is shown by the fact that a few

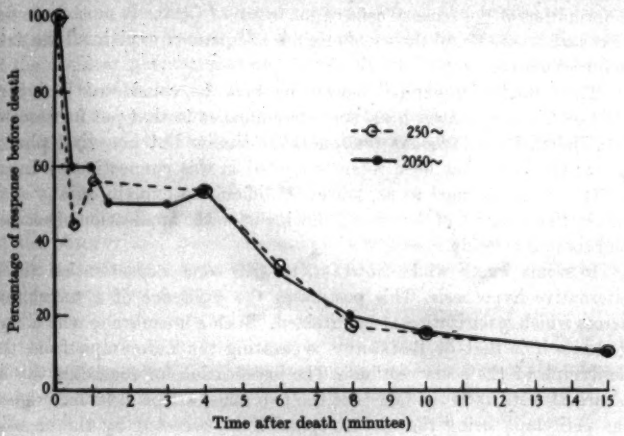

Fig. 3.

crystals of sodium chloride similarly applied have an even more rapid and profound effect in causing the disappearance of the phenomenon.

(5) It has been suggested to us that, after death of the animal, the response to tones of high frequency suffers a larger decrement than does that to tones of low frequency. We have not, however, found this to be the case. The response to tones of 250 and 2050 cycles at various intervals after death has been plotted in the form of a graph in Fig. 3. The response is plotted as a percentage of that before death, both frequencies being reduced to 100 p.c. at time 0.

As may be seen, the small disparities existing are well within the experimental error to be expected.

Discussion.

It must be stressed that none of the experimental work adduced above is conclusive in differentiating between the neural and non-neural hypotheses of this effect. Nevertheless, considerable evidence exists against theories in the former category, for some discussion of which reference may be made to a recent paper by Davis and others [1934], in which a non-neural theory is put forward; this states, briefly, that the potentials constituting this effect arise as a result of the mechanical deformation of the sensory cells of the organ of Corti. It must be noted that such a non-neural theory of origin is adequate to explain all the data adduced above.

The "fluid-microphone" suggestion may be considered [Adrian, 1931 *a*]. This hypothesis bears some resemblance to that put forward by von Helmholtz to account for somewhat similar but non-vital phenomena; the latter has been recently quoted in this connection by Leiri [1934]. It has seemed to us, however, difficult to explain on any such theory the cessation of the effect following upon the application of cocaine and sodium chloride.

It seems worth while, however, to give some consideration to an alternative hypothesis. This postulates the existence of a membrane across which polarization is maintained. Such a membrane which suggests itself is that of Reissner, separating the endolymph from the perilymph of the scala vestibuli. The justification for regarding this as polarized rests upon the fact that the two fluids are of different origins, the perilymph being the cerebro-spinal fluid, secreted by the choroid plexuses, and the endolymph being secreted within the endolymph system itself, possibly by the stria vascularis. The fact that these fluids are of different origins renders possible the hypothesis that they are of different ionic concentration [*vide* Rossi, 1914], and the assumption not unduly heroic, that this would lead to polarization of an intervening semi-permeable membrane, such as that of Reissner.

The other possibility of localizing possible polarization planes within the cochlea is presented by the tectorial membrane. This may be regarded as a membranous structure, filled with a gelatinous substance possibly produced by the vital secretory activities of the epithelial cells of the limbus, whence it arises.

Whichever of the two possibilities be adopted in the localization of a polarized structure within the cochlea the essential features of the hypothesis remain the same; namely, that the electrical phenomena of the

cochlear effect are engendered by pressure waves, which in turn are the result of the vibrations set up as a result of sound stimulation, producing movements of such a membrane.

Since the electrical effects would be maximal in the region of maximal amplitude of movement, and since the maintenance of polarization must be a vital phenomenon dependent upon secretory activity, it is readily understandable how, accepting the resonance hypothesis, the effect is in the first place greatest for high frequencies at the base of the cochlea, and at the apex for low, and why, secondly, considerable weakening of the effect is so speedily consequent upon death of the animal, or failure of the auditory circulation. The adoption of any non-neural explanation of the cochlear phenomenon has inevitably to leave as yet unanswered the problem of its biological significance. Whether it should be regarded as being of an epiphenomenal nature or alternatively as constituting the adequate stimulus of the auditory end organs are questions which remain open.

Summary.

The results of an experimental investigation of some of the attributes of the Wever and Bray effect in the cochlea are described. Details of the experimental technique, amplifying and recording (cathode-ray oscillograph) system are given.

Evidence is put forward upon the following points:

(1) Cessation of the effect upon section of the eighth nerve. That this is due as suggested by Adrian to concomitant severance of the internal auditory artery is given precise confirmation by the persistence of the effect, when section of the nerve is carried out with preservation of the vessel.

(2) The relative amplitude of the potentials developed in response to tones of high and low frequency, between an indifferent electrode in the neck muscles and a differentiated electrode making contact with:

(*a*) Various points upon the surface of the cochlea.

(*b*) Various points within the cochlea.

The evidence adduced indicates firstly the good electrical insulating properties of the bony capsule of the internal ear, and secondly that the responses from the apical and basal parts of the interior of the cochlea show marked differentiation for frequency, the low tone response being accentuated at the apex and the high tone response at the base.

This is considered by the writers to constitute strong evidence of a direct nature in favour of the hypothesis that the cochlear elements are differentially tuned.

(3) The highly sinusoidal nature and serial uniformity of the cochlear response over certain frequency ranges are indicated.

(4) The application of crystals of sodium chloride to the round window membrane is found to cause an even more rapid disappearance of the response than the application of cocaine crystals.

(5) Following death, the response displays a parallel decrement for high and low frequencies.

Although none of the evidence derived from these experiments is considered decisive in assigning to the cochlear effect a neural or non-neural origin, the balance of the evidence is thought to favour a non-neural hypothesis. Such hypotheses already put forward are considered, and an alternative suggestion, based upon the probable presence in the cochlea of a polarized membrane, is advanced.

We are greatly indebted to Mr D. W. Ashcroft for considerable assistance in carrying out many of the experiments.

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612.352.2:547.435

CHOLINE AND THE DIETARY PRODUCTION OF FATTY LIVERS.

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(Received March 1, 1934.)

The production of fatty livers in rats by the administration of a diet consisting of mixed grain and 40 p.c. of beef fat has been demonstrated by Best, Hershey and Huntsman [1932]. The work of other authors has shown that fatty livers may be produced by feeding animals on diets containing cholesterol. Thus the results of Chalatow [1912], Chalatow and Anitschow [1913], Bailey [1916], McMeans [1915], Yuasa [1928], Kimura [1931] all show directly or indirectly that diets containing cholesterol cause a change in the nature or an increase in the amounts of the liver lipoids. More recently Okey [1933*a*] showed that the dietary administration of 1 p.c. of cholesterol to rats caused a large increase in the lipoid content of the liver, and in particular a remarkable rise in the amount of total cholesterol, far the greater part of which was present as cholesteryl esters. Similar results have been reported by Chanutin and Ludewig [1933]. Thus fatty livers may be produced either by feeding to animals certain diets of high fat content or by inclusion in the diets of a relatively small amount of cholesterol, methods which are far less unphysiological than the action of the drugs commonly used for this purpose. Their importance is emphasized by the finding that the inclusion of choline in the diets in the relatively small amount of about 1 p.c. prevents the appearance of the fatty liver which they would otherwise cause [Best and Huntsman, 1932; Best and Ridout, 1933]. Dietary methods are now therefore available, both for producing fatty livers and for preventing them by the use of substances, sterols, fats and phosphatides, which are normal constituents of both plant and animal cells. Further, although the amounts in which these substances have been experimentally employed may be greater than are usually

present in a diet, they are such as to lead one to believe that the mechanism involved may perhaps finally prove to be one of those which occur in the normal animal, and which have come to light because of the unbalancing by experiment of two factors which are antagonistic.

Best and Huntsman [1932] and Best and Ridout [1933] in investigating this action of choline used the Liebermann saponification method as modified by Leathes and Raper, but without removal of unsaponifiable matter, for the determination of the amount of "fat." Since the figures obtained by this method give merely the sum of the total fatty acids and the unsaponifiable substances, their results, although effectively demonstrating the preventive action of choline, provided no evidence of the changes undergone by the individual components of the "fat." The subsequent publications of Okey [1933 *a*, *b*] showed that the fatty liver induced by cholesterol is characterized by the appearance of large amounts of cholesteryl ester. It became of importance, therefore, to determine whether the fatty liver caused by the feeding of diets high in fat bore any relation to that caused by cholesterol feeding. This was necessary in order further to investigate the action of choline in preventing fatty livers from both these causes, since choline might be preventing the occurrence of the fatty liver because it was involved in the metabolism either of glycerides or of cholesterol, or possibly of both. Because choline occurs in the tissues generally as lecithine and sphingomyeline, it was also important to determine whether the amounts or nature of these substances in the tissues was modified by adding choline to the diet. We have therefore carried out a comprehensive study of the effects of various diets on the nature and the amounts of the individual constituents of the lipoids of different tissues of groups of rats, and at the same time have attempted to investigate the modifications of these constituents caused by giving to other groups of animals the same diets supplemented by choline. Three diets have been employed. Of these, two have been such as to cause fatty livers, one containing a high percentage of fat, the other added cholesterol, whilst the third has been an ordinary stock diet which results in no accumulation of fat in the liver. In these investigations the total ether-soluble material of the livers, hearts, lungs, spleens, kidneys and carcases of groups each of twenty to thirty animals, on the three diets, with and without added choline, has been investigated. The tissues from each group have been pooled in order to permit of the investigations being as complete as possible, and because the very large number of determinations on individual livers already carried out has shown that both the high fat diet and that containing cholesterol will

cause intensely fatty livers and that choline added to these diets prevents this. In the present paper we record only the results which have been obtained on the livers, because they seem to us to advance the subject sufficiently to warrant their publication at the present stage in view of the delay which the tedious nature of the investigation of the other tissues entails.

Methods.

The diets used were based on the grain diets previously employed (32·7 p.c. each of oatmeal, cornmeal and cracked wheat with 1·9 p.c. of bone meal), modified for the different experiments as set out in Table I, where the other relevant figures are recorded.

Table I.

Diet	No. of rats	Average weight before exp. g.	Average weight after exp. g.	Average difference in g.	Duration in days	Average food consumption per day g.
F: grain 60 p.c., beef dripping 40 p.c.	20	188·2	193·9	+5·7	23	7·8
G: as F, with 0·1 g. choline chloride per rat per day	20	188·3	195·2	+6·9	23	8·9
H: grain 80 p.c., "Crisco" 20 p.c., cholesterol 2 p.c.	29	169	165	−4·0	26	9·1
J: as H, with 0·23 g. choline chloride per rat per day	29	175	169	−6·0	26	9·4
L: grain	20	195·5	205·1	+9·6	21	11·0
M: grain, with 0·1 g. choline chloride per rat per day	20	191·7	205·2	+13·5	21	12·5

The animals were killed by a blow on the head 18 hours after their last meal, and their livers removed.

The weighed livers were minced with sand immediately under five volumes of absolute alcohol. The mixture was heated to boiling and filtered. Two further treatments with hot alcohol were then given, followed by one with ether. At this stage the material was reground and further extracted three times with hot ether. The combined alcohol-ether extracts were evaporated to dryness *in vacuo*, and the residue extracted with warm ether several times and the mixture filtered. Such an extract will, of course, contain large amounts of materials not of lipoid nature, nor soluble in pure ether, but soluble in ethereal solutions of phosphatides such as these. The combined ethereal extracts were concentrated and transferred to a standard flask, from which suitable aliquots were withdrawn for analysis. The unsaponifiable fraction and

fatty acids were prepared as described by Channon and El Saby [1932]. Free cholesterol and total cholesterol were estimated by the digitonin method on the original material and on the unsaponifiable fraction respectively. Ether-soluble phosphorus was determined by the colorimetric method after perhydrol oxidation. The total choline in the extract was assayed on the rabbit's intestine after conversion to acetylcholine.

After determination of the required fat constants, the remainder of the solution was evaporated to dryness. The residue was dissolved in four volumes of ether and precipitated by excess of acetone at room temperature. After standing for some hours, the mixture was filtered, and the residue, after washing with acetone, was redissolved in 25 p.c. solution in ether and reprecipitated by acetone. After filtration, the phosphatide was dissolved in ether and its weight obtained after evaporation to dryness. The fat fraction contained in the acetone ether mother liquors was also weighed. These two fractions were then hydrolysed by boiling with a solution of sodium ethylate in absolute alcohol. From the fat fraction the unsaponifiable fraction was removed after hydrolysis and the fatty acids prepared. The fatty acids were also prepared from the phosphatide fractions. Iodine values [Rosenmund and Kuhnhenn, 1923] were determined, and a separation into solid and liquid acids by the Twitchell process carried out in some cases, with subsequent determination of the iodine values of the solid and liquid acids.

Results.

In Table II are recorded the weights of the livers of each group of experimental animals and the percentage yield of extract, together with

Table II. Weights of livers, yield and percentage composition of extracts.

	F	G	H	J	L	M
Wt. of livers, g.	135	133·8	246·2	220·5	132·5	132·5
Wt. of extract, p.c.	16·86	6·46	17·93	9·40	5·42	5·27
The extract contained, p.c.:						
Fatty acids	82·77	46·67	70·44	46·60	50·99	48·98
Unsaponifiable matter	5·55	9·42	18·48	18·44	7·99	6·25
Free sterol	1·31	3·04	1·61	2·91	3·57	3·80
Total sterol	1·87	3·17	16·02	14·10	4·24	3·95
Ester sterol	0·56	0·13	14·41	11·19	0·67	0·15

its percentage composition; in Table III the quantity of certain components in 100 g. of liver is given.

The method of calculating the amount of the various fatty constituents of the liver, which are shown in Table III, needs a word of explanation.

TABLE III. Yields per 100 g. of fresh liver.

	F	G	H	J	L	M
Total fatty acids	13·96	2·98	12·63	4·38	2·76	2·58
Neutral fat	12·76	1·09	9·50	1·51	0·40	0·27
"Lecithine"	2·39	2·76	2·36	3·08	3·36	3·29
Total sterol	0·315	0·206	2·872	1·323	0·230	0·205
Free sterol	0·221	0·196	0·289	0·273	0·194	0·197
Cholesteryl oleate	0·158	0·020	4·350	1·768	0·036	0·008

Groups G, J and M received choline.

The figures for "lecithine" given in that table have been calculated from the estimation of phosphorus in the original ether extract upon the arbitrary assumption that all the phosphorus was present as dioleyl lecithine (P 3·86 p.c.). Calculations have also been made from the yield of fatty acids on hydrolysis of the crude acetone precipitate upon the same assumption. From comparison of the results obtained by these two methods it appears that in the case of F and H all the phosphatide has been precipitated by acetone, but that in the case of G, J, L and M some phosphatide has remained in the mother liquor, for the figures are 11, 17, 9 and 6 p.c. lower respectively than when calculated by the former method. This inference is confirmed when the calculation is made from the phosphorus content of the fractions into which the original ethereal extracts were separated. It is immaterial which figure is taken inasmuch as the effect on the figure for neutral fat is less than 0·2 p.c. of the liver weight and accordingly the figure based on the ether-soluble phosphorus has been used in the calculations in Table III.

The figures for neutral fat have been obtained by subtracting from the total fatty acids those present as "lecithine" (70·4 p.c. oleic acid) and as cholesteryl ester $\left(\text{oleic acid} \times \frac{282}{650}\right)$, and multiplying the difference by 1·045 to convert oleic acid into triolein.

DISCUSSION.

(1) *The effects of high fat and cholesterol feeding.*

In discussing these results, the figures for group L, which was the only group receiving a normal diet, may be taken as the standard. If we confine our attention to the two groups F and H which received diets high in fat and containing added cholesterol respectively, it is seen that although the combined figures for the fatty acids and unsaponifiable matter which would have been obtained by the Liebermann saponification method yield very similar total figures, *i.e.* 14·9 and 15·97 p.c. respectively, these two means of producing fatty livers have given a

markedly different distribution of the constituents. High fat feeding in the conditions of these experiments has caused something like a 25 p.c. greater rise in neutral fat than has cholesterol administration. This large increase in neutral fat has resulted in an insignificant rise in free sterol (cf. F 0·221 with L 0·194) and a small but definite rise in cholesteryl ester (cf. F 0·158 with L 0·036). In the cholesterol fed series H, however, the most striking effect in spite of the large increase in neutral fat is the increase of cholesterol oleate from 0·036 in the control series L to 4·35 p.c. There is also an increase in free cholesterol to 0·289 p.c. which although of smaller degree seems to be significant. It is not easy to interpret these different types of fatty liver. It rather looks as though the accumulation of free cholesterol in the liver was harmful and that its presence in abnormal amounts there caused a mobilization of neutral fat; such a mobilization might be of service for the conversion of cholesterol into its fatty acid esters and so lowering its melting point. In any case it appears to be significant that when cholesterol is fed to animals it does not appear in the liver in the free form in any greatly increased amount.

(2) *The effect of choline.*

(*a*) *Neutral fat.* The effect of choline administered with high fat diet has been to reduce the glyceride fraction from F 12·76 p.c. to G 1·09 p.c., and with the cholesterol diet from H 9·50 p.c. to J 1·51 p.c. The livers of the animals receiving the stock diet (L) contained 0·40 p.c. of neutral fat, and if this be taken as the control value, it is seen that although the daily amounts of choline chloride administered to the animals of these groups, G 0·1 g., J 0·23 g. respectively, have in very large measure prevented the accumulation of neutral fat, they have not quite caused a normal value. The clear deduction can be made, however, that choline administration prevents the appearance of neutral fat in the liver in diets which would otherwise cause fatty livers. Even in series M the neutral fat fraction (0·27 p.c.) is less than that of L (0·40 p.c.) by an amount which we are inclined to believe may be significant. If this be so, it seems forcibly to suggest that one very definite effect of choline at least is to decrease the neutral fat fraction of the liver, irrespective of the amount in which it would otherwise occur.

(*b*) *Cholesterol and cholesteryl esters.* Choline has no effect on the free sterol of the livers of any of the groups. The slight fall in G (0·196 p.c.) from F (0·221 p.c.) is probably merely incidental to the fall in the neutral fat fraction. The effect on the ester fraction is, however, marked as is seen by comparing G 0·020 p.c. with F 0·158 p.c., J 1·768 p.c. with

H 4·35 p.c. and M 0·008 p.c. with L 0·0..6 p.c. The most striking feature of these results is that whereas choline has prevented the increase in the neutral fat fraction of the livers of the animals receiving cholesterol as effectively as it has done for those receiving the high fat diet, it has failed to prevent more than 60 p.c. of the very large rise in the cholesterol ester fraction which the former diet has caused, 1·768 p.c. in group J compared with 4·350 p.c. of series H, and 0·036 p.c. of the control series L.

Blatherwick, Medlar, Bradshaw, Post and Sawyer [1931, 1932, 1933 *a*, *b*] have produced evidence that feeding animals on whole dried liver, raw liver or certain liver extracts causes fatty livers in rats. They found that the addition of 2 p.c. of lecithine to the diet did not prevent the appearance of these fatty livers, and they contrast their results with those of Best and his colleagues. We believe that the explanation of their contradictory results may lie, in part at least, in the relatively small amount of lecithine administered. Thus the dried liver diet, as well as a number of other diets used by Blatherwick and his co-workers contain not only enough fat to cause a very fatty liver but also enough cholesterol. Hence the addition of 2 p.c. of lecithine to the diets used by them, *i.e.* the ingestion of 30 mg. of extra choline daily by each rat, is an amount which would not be expected to be effective, because it has to oppose the combined effects of the two different factors in their diets, each of which can independently cause fatty livers, since each rat receiving the dried liver diet ingested 4·16 g. of fat and 87 mg. of cholesterol daily. We shall defer further comment on the results of Blatherwick until certain additional experimental work has been completed.

(*c*) *The phosphatide fraction.* The effect of choline administration is best seen from Table IV, in which the figures for ether-soluble phosphorus are compared with the figures for the sum of the total fatty acid and total cholesterol contents arranged in descending order of magnitude.

TABLE IV. In 100 g. liver.

	H	F	J	G	L	M
Sum of total fatty acids and cholesterol, g.	15·50	14·27	5·70	3·19	2·98	2·78
Total fatty acids, g.	12·63	13·96	4·38	2·98	2·76	2·58
Ether-soluble phosphorus, mg.	91·1	92·3	119·0	106·5	129·7	127·0

Groups G, J and M received choline.

These figures confirm existing knowledge that the percentage of phosphatide in general falls with fatty infiltration although the results with groups G and J are somewhat anomalous. They show that the percentage of phosphatide is greater in the livers of the groups which

received choline G and J, compared with the corresponding control groups F and H. On the other hand, choline administration has not affected the phosphatide percentage of group M. Bearing in mind that the water content of livers is reduced by fat infiltration, the phosphatide increases in groups G and J are actually much greater when referred to dry weight than appears when the phosphatide is expressed as percentage of the fresh tissue as in Table IV. The lack of increase in group M compared with group L makes interpretation of these phosphatide changes difficult. It may suggest that the effect of choline in increasing the phosphatide percentages in groups G and J over groups L and M is possibly only an indirect one resulting from the action of choline in preventing the fatty liver.

(*d*) *The fatty acids.* The iodine value of the fatty acids of the beef fat used for groups F and G was 40·4, and of the "Crisco" used for H and J 65·8. The stock diet of grain contained very little fat and its nature was not investigated. In Table V are recorded the iodine values of the fatty acids of the fat and phosphatide fractions, together with those of the solid and liquid acids of the latter, determined by the Twitchell method.

TABLE V.

	Iodine values of fatty acids		Phosphatide acids		Iodine values	
	Fat fraction	Phosphatide fraction	% solid	% liquid	Solid	Liquid
F	73·8	135	36·6	63·4	14	219·1
G	72·6	130·7	41·0	59·0	9	205·3
H	90·0	130·2	35·8	64·2	19	196·3
J	87·5	124·4	lost	61·7	21	176·2
L	92·0	—	42·6	57·4	16	186·2
M	88·4	—	43·1	56·9	12	179·5

The general deduction to be made from these figures is that choline administration has materially affected neither the distribution of the fatty acids nor their iodine values. The iodine values of the fat fractions of F and G, and of H and J, reflect the influence of the dietary fats. The other variations which appear in the iodine values, such as that of the phosphatide fatty acids of J, 124·4, and in the relatively high values of those of the solid fraction of the phosphatide acids and the low value of the liquid acids of the phosphatide in J and M, we believe to be occasioned by unavoidable delays in working up of the small amounts of material available, and to be regarded as of no significance.

(*e*) *The choline content of the livers.* Determinations were made of the choline in the total extracts and in the protein residues of the livers by biological assay on the rabbit's intestine after conversion to acetylcholine.

These investigations, in which Mr J. P. Fletcher has taken a considerable part, will be described in detail in a subsequent communication, in which the question of the amounts of free and combined choline present in tissues will be fully discussed. Acid hydrolysis of the protein residues and subsequent assay following conversion to acetylcholine showed that the amount of choline in the residues of F, G, H and J was on the average only 2·4 p.c. of the total choline in the ethereal extracts prepared as described (extreme values 4·1 and 1·4 p.c.). Further, the total choline content of the phosphatide precipitates averaged 92·4 p.c. of that of the ethereal extracts from which they had been obtained (extreme values 87·3, 98·1 p.c.). Attempts to determine the free choline in the ethereal extracts gave an average value of 4·92 p.c. of the total (extremes 4·61, 5·82 p.c.), and a similar small average figure for free choline, namely 5·5 p.c. of the total, was found in the phosphatide precipitates. We thus incline to the view that it is probable that free choline is not present in liver in significant amounts, and that the amounts found have been produced by slight hydrolysis of the phosphatides present in the extracts. For the purposes of discussion, therefore, it appears legitimate to regard our extracts as having contained all the choline of the original tissues, and further that this choline is entirely present as phosphatide, for the small amount not present in the phosphatide fraction (7·6 p.c. of the total) is explained by the fact that not all the phosphatide was precipitated by acetone as explained on p. 414. It is therefore possible to compare the effect of choline on the nature as opposed to the amount of the phosphatide fraction, and to determine whether its administration has affected the amounts of lecithine or sphingomyeline at the expense of the kephaline and the other unknown constituents of liver phosphatides. In Table VI are recorded the total amounts of choline found, the choline calculated on the basis that the ether-soluble phosphorus is entirely present as lecithine, and the ratio of the choline found to the latter figure.

TABLE VI.

	F	G	H	J	L	M
(1) mg. P/100 g. liver	92·4	106·6	91·2	119·0	129·5	127·0
(2) Choline $P \times \frac{121}{31}$ mg.	354·6	409·2	349·8	456·6	497	487·5
(3) Choline found	177	219	201	252	266	281
Choline found as a percentage of (2)	49·4	53·5	57·45	55·2	53·5	57·6

Groups G, J and M received choline.

Comparison of G with F, and J with H shows that choline administration has not affected the proportions in which the choline containing

phosphatides occur in the general phosphatide mixture. The figures for M show a greater divergence from the control series L, but it appears tolerably certain that this difference is not outside the experimental error. We incline to the view, therefore, that the composition of the phosphatide mixture is unaltered by the administration of choline.

Conclusions.

These findings therefore show essentially that the inclusion of suitable amounts of choline in the diet used will prevent that occurrence in the liver of the abnormal amounts of neutral fat and in part of the cholesteryl esters, which would otherwise result. It is early yet to form a view as to the mechanism of this action. Previous work in the Toronto Laboratories has shown that the fat excretion of animals on a high fat diet is not increased by choline administration and therefore neither the total absorption nor the excretion is involved. The results on the other tissues, so far as they are complete, suggest that the effect of choline is on the liver only, and for the purposes of discussion this will be considered as if it were proven. The rôle of the liver in the metabolism of fat is little understood beyond the essential fact that the liver appears of prime importance in this connection, as is shown by the mobilization of fat to it caused by the action of various drugs and pathological conditions and its ability selectively to absorb unsaturated acids from the blood after fat absorption. In the case of diets of high fat content the effect of choline might be concerned either with the transport of fatty acids from the liver or directly or indirectly with their oxidation. With the fatty livers induced by the cholesterol feeding the former hypothesis might be applicable, while the catabolism or excretion of cholesterol might also be involved.

The possible function of lecithine in the transport of fatty acids from the liver to the tissues for oxidative purposes has often been discussed, especially in the light of the hypothesis of Leathes that one of the functions of the liver may be the preliminary preparation of the fatty acids for more ready use by the tissues by rendering them less saturated. If lecithine does play an active rôle in fat transport, it is conceivable that the provision of extra choline in a diet containing much fat might explain the preventive action of choline. On the other hand, there is no obvious reason why fat should accumulate in the liver on a high fat diet. Further, the action of choline in preventing the "cholesterol" fatty liver is perhaps unlikely to be concerned in a mechanism involving lecithine as a carrier of fatty acids. These two types of fatty liver involve essentially two dif-

ferent substances, the fatty acid and cholesterol, and it seems reasonable to seek an explanation of the action of choline in a common process in which both may be concerned. The most probable suggestion appears to be that choline in some way or other accelerates either directly or indirectly the oxidation of fatty acids and cholesterol by the liver itself, and to this problem we are giving our attention.

But apart from speculations, the fact is established that even though the fatty liver contains its normal amount of choline as phosphatide it is yet fatty, and therefore if choline should prove to be normally involved in fat removal either directly or indirectly this implies that little of the liver phosphatide may be called upon to supply choline. Such a result is to be expected, because the studies of the French school of Mayer, Schaeffer, and Terroine have repeatedly shown that even in death from inanition, the phosphatides of such tissues as liver cannot be called upon for energy requirements. A further point emerges also. If choline provides in any way a means whereby fat infiltration in the liver is normally controlled, the occurrence of fatty livers produced by diet implies a limited ability of the animal to synthesise the base for this purpose. Apart from fat oxidation, choline might be counteracting some effect of fat infiltration in interfering with the formation or oxidation of carbohydrate by the liver, and in this connection the results of Best and Hershey [1932] need consideration. These authors showed that when lecithine was given to depancreatized dogs which were showing the signs of failure of liver function, the urinary sugar excretion markedly increased. If carbohydrate formation or oxidation were involved, the removal of the two types of fatty liver by choline might result in this indirect manner.

These results also show that choline is far more active in preventing the accumulation of neutral fat in the fatty livers than of cholesterol. This is seen in the F and G and the H and J series. In G 0·1 g. of choline chloride per day has reduced the neutral fat fraction from 12·76 to 1·09 p.c., a reduction of 11·67 g./100 g. liver; in J 0·23 g. of choline chloride has reduced it by 7·99 p.c., approximately to the same level as that of G. From the results with G this fall in neutral fat by 7·99 p.c. would require less than 0·1 g. of choline chloride. The J series received, however, 0·23 g., and this increased amount has merely resulted in the removal from the liver of 2·58 g. of cholesteryl ester, leaving still in the liver 1·768 g. It must not be overlooked, however, that the effect of choline may be on the fatty acids of the neutral fat only. Cholesterol may accumulate as ester only in the liver for reasons which have been mentioned on p. 414. If this were so, and if choline affected the oxidation or

translocation of the neutral fat fraction only, possibly the removal of that fraction would entail the disappearance by excretion of the cholesterol esters.

Summary.

1. Three diets have been given for periods of 21–26 days to groups each of 20 or 30 rats. These diets have been (*a*) a diet containing 40 p.c. of fat; (*b*) one containing 2 p.c. of cholesterol; (*c*) the normal stock diet. In three other groups the animals have received similar diets over a similar period, but each rat has had also 0·1–0·23 g. of choline chloride daily.

2. The total lipoid material from the pooled livers of each group has been prepared and analysed for its content of free and combined sterol, neutral fat, phosphatide and choline.

3. The fatty liver caused by high fat feeding is occasioned entirely by increase in the neutral fat fraction. That caused by cholesterol feeding is characterized by the appearance of excessive amounts of cholesteryl esters, together with a large increase in neutral fat. The phosphatide content of both these types of fatty livers varies inversely with their total lipoid content.

4. Choline administration prevents the appearance of both types of fatty liver, from which it is deduced that choline is concerned in the metabolism both of neutral fat and cholesterol.

5. The percentage of phosphatide in the livers is increased by choline administration probably indirectly and because choline prevents the occurrence of the fatty liver.

6. Estimations of choline by biological assay after conversion to acetylcholine show that choline administration does not increase the proportions in which the phosphatides containing choline (lecithine and sphingomyeline) occur in the phosphatide mixture.

7. Choline administration has had no significant effect on the degree of unsaturation of the fatty acids of the fat or phosphatide fractions.

8. The possible mechanism by which choline exerts its effect on the amount of the liver lipoids is discussed.

We wish to express our gratitude to Dr J. G. FitzGerald, Dean of the Faculty of Medicine in the University of Toronto, for his kind interest in this work.

We also wish to record our thanks to Mr J. Truax for his valuable technical assistance.

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THE ACTION OF SOME AMINES RELATED TO ADRENALINE, METHOXYPHENYL-METHOXYETHYLAMINES.

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(*Received March* 13, 1934.)

In a previous paper in this *Journal*, Epstein, Gunn and Virden [1932] described the actions of four derivatives of phenylethylamine, viz. (1) *p*-methoxy-, (2) *m*-methoxy-, (3) methylene-dioxy- and (4) dimethoxy-phenylethylamines.

The approximate minimum lethal dose in g./kg. for mice by intraperitoneal injection was found to be as follows: (1) 0·15, (2) 0·23, (3) 0·30, (4) 0·42. The first three stimulated, the fourth depressed, the central nervous system. The first three stimulated the sympathetic terminations in the cat but not in rodents; the fourth had no sympathomimetic action in any of these animals. All four compounds had a direct stimulant action on smooth muscle in rodents. In the decerebrate cat, compounds (1) and (2) had about 1/300, compound (3) about 1/400, of the pressor activity of adrenaline.

It was surprising to find that dimethoxyphenylethylamine, which approximated most closely to adrenaline in structure (in so far as it possessed two phenolic methoxyl groups in the same relative position as the phenolic hydroxyl groups in the adrenaline molecule), deviated farthest from adrenaline in physiological action.

Almost simultaneously with the appearance of their paper, Raymond-Hamet [1932 *a*] published an account of the actions of various methoxy-phenylethylamines. His experiments were made chiefly on dogs, anæsthetized by chloralose, with both vagi cut and under artificial respiration. The results he obtained were, considering the dissimilarity in the animals used and in the conditions of experiment, in close agreement with theirs, in so far as the two sets of experiments were parallel.

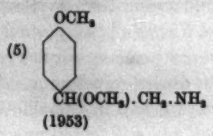
Raymond-Hamet found that paramethoxyphenylethylamine produced a rise of blood-pressure with inhibition of intestinal movements in doses of 1–10 mg./kg. It possessed about 1/150 to 1/200 of the pressor activity of adrenaline, but the rise of blood-pressure was more prolonged. Epstein, Gunn and Virden found the same result in cats, but estimated the pressor activity at about 1/300 of adrenaline.

Metamethoxyphenylethylamine was almost equal in pressor activity to the para compound, while the ortho compound was much less active. He found that the 3-4 dimethoxy-compound was only feebly pressor in small doses and produced a prolonged fall of pressure in larger doses; this agreeing also with their results in cats with this compound. The only point of difference was that he found inhibition of intestinal movements in dogs whereas they found augmentation in cats.

Methylation of phenolic hydroxyl groups in this type of compound produces, therefore, very considerable changes, quantitative and qualitative, in physiological action.

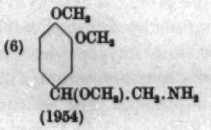
Dimethoxyphenylethylamine comes very close in structure to the alkaloid, mescaline, which is 3 : 4 : 5 trimethoxyphenylethylamine. It was interesting to pursue further the effects of methylation on compounds of this type. Dr Guggenheim kindly sent one of us several such compounds. The actions of some of these have been investigated in this laboratory by Grace [1934]. He found that mescaline had no sympathomimetic action, as had been previously found by Raymond-Hamet [1932 *b*]. It had a purely depressant action on the central nervous system, and closely resembled dimethoxyphenylethylamine qualitatively in its actions. It was slightly less toxic for mice by intraperitoneal injection, 0·55 g./kg. as compared with 0·42.

In the present paper we wish to consider the effect of introduction of a methoxyl group in the ethylamine side-chain. The following three compounds have been investigated:



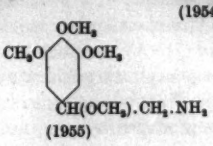
(5)

(1953)



(6)

(1954)



(7)

(1955)

The numbers underneath the formulæ are the original serial numbers of the amines as received from Dr Guggenheim and are mentioned because these numbers appear on many of the illustrations.

Toxicity. Table I gives a comparison of the approximate minimum lethal doses by intraperitoneal injection for mice of six related compounds:

(1) *p*-methoxyphenylethylamine.
(4) 3-4 dimethoxyphenylethylamine.
(*M*) 3:4:5 trimethoxyphenylethylamine (mescaline).
(5) *p*-methoxyphenyl-methoxyethylamine.
(6) 3:4 dimethoxyphenyl-methoxyethylamine.
(7) 3:4:5 trimethoxyphenyl-methoxyethylamine.

TABLE I. Minimum lethal dose for mice by intraperitoneal injection, g./kg.

Compound	Minimum lethal dose	Compound	Minimum lethal dose
(1) (CH_3O) PE	0·15	(5) (CH_3O) P—(CH_3O) E	0·35
(4) $(CH_3O)_2$ PE	0·42	(6) $(CH_3O)_2$ P—(CH_3O) E	0·50
(*M*) $(CH_3O)_3$ PE	0·50	(7) $(CH_3O)_3$ P—(CH_3O) E	0·50

P = phenyl; E = ethylamine.

The left vertical column shows that the successive additions of methoxyl groups to the ring (the ethylamine side-chain remaining unchanged) cause a progressive diminution in toxicity. This is true also, as is shown in the right vertical column, if the side-chain contains a methoxyl group in the β position. The diminution in toxicity is greater in proceeding from one methoxyl group to two, than from two to three, and, as was shown in our previous paper, the former change involves otherwise also a greater change in physiological activity.

A comparison of the horizontal pairs in the above table shows that the addition of a methoxyl group to the ethylamine side-chain in the β position produces also, if anything, a diminution in toxicity for mice.

Symptoms produced. Epstein, Gunn and Virden [1932] found that the monomethoxyphenylethylamines produced a primary stimulation of the central nervous system in mice, whereas the dimethoxy-compound was purely depressant. The addition of a methoxyl group in the side-chain further diminished the stimulant action which was still feebly present in (5) but absent in (6) and (7). All compounds eventually produced motor paralysis with death from respiratory failure preceded by weak asphyxial convulsions. Compound (5) produced primary twitches similar to, but less intense than, those produced by compound (1).

Action on the isolated mammalian heart. It was shown in the previous paper that *p*-methoxyphenylethylamine produced a characteristic sympathomimetic action on the cat's heart, but no such effect on the heart of the rabbit. Compound (5), however, produced merely depression of the hearts both of cats and rabbits. Fig. 1 shows this depressant action produced by a concentration of 1 in 20,000. A slightly greater slowing and weakening of the contractions was produced by a further four minutes' perfusion. When the drug solution was replaced by Locke's solution the heart promptly recovered. The heart was then perfused with compound (1), which produced a typical augmentation and acceleration.

Fig. 1. Isolated perfused cat's heart, at ↑ *p*-methoxyphenyl-methoxyethylamine, 1 in 20,000. Showing absence of sympathomimetic action.

Fig. 1 may be contrasted with Fig. 1 in the previous paper. Six experiments were done on the cat's heart with compound (5) and with concentrations varying from 1 in 10,000 to 1 in 50,000, but in no case was any sympathomimetic effect obtained. So far as the cat's heart is concerned, therefore, the addition of a methoxyl group to the side-chain of *p*-methoxyphenylethylamine has removed the sympathomimetic action. Compound (5) had merely a depressant action in high concentration on both the rabbit's and the frog's heart.

Compounds (6) and (7) also showed merely depressant actions on the heart.

Action on blood-pressure. (*a*) *Cat.* In the cat anæsthetized with a mixture of chloroform and ether and with the central nervous system intact, a dose of 0·004 g./kg. intravenously of compound (5) gave a rise

of blood-pressure of 40 mm. Hg, with a slight diminution of respiratory movements; a dose of 0·01 g./kg. (Fig. 2) gave a transient fall of blood-pressure of about 20 mm., with a more pronounced reduction in the amplitude of the respiratory excursions. Larger doses produced an increasingly greater fall of pressure.

Under the same circumstances compound (6) gave a smaller rise of blood-pressure, 0·004 g./kg. giving a rise of 10 mm. Hg, 0·01 a slight rise

Fig. 2. Uterine movements (*U*), intestinal movements (*I*) (contractions recorded upwards), respiratory movements (*R*) and blood-pressure (*B.P.*) in a cat anæsthetized by ether and chloroform: *p*-methoxyphenyl-methoxyethylamine, 0·01 g./kg. intravenously.

or sometimes a slight fall (Fig. 3). Compound (7) gave a slightly more pronounced fall (20 mm. Hg with a dose of 0·01 g./kg.).

In the anæsthetized cat, therefore, all three compounds seemed to act qualitatively alike on blood-pressure, though compound (5) is more active than the other two.

In the spinal (decapitated) cat compound (5) produced a very marked rise of blood-pressure. In one experiment a dose of 0·003 g./kg. gave a rise of 62 mm. Hg, in another 0·01 gave a rise of 126 mm. Hg. A dose of 0·004 g./kg. of compound (1) gave a rise of 126 mm., as compared with

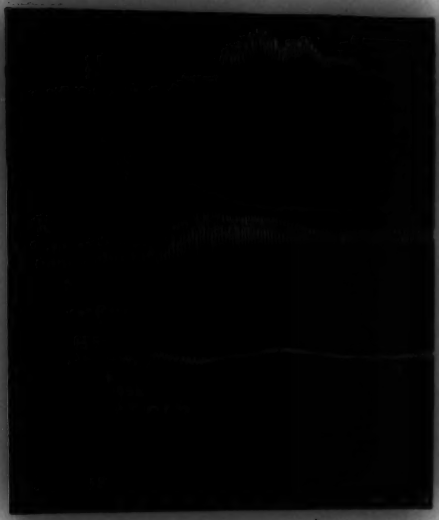


Fig. 3. Records as in Fig. 2. Effects of dimethoxyphenyl-methoxyethylamine, 0·01 g./kg. intravenously.

Fig. 4. Blood-pressure in a decerebrate cat. Comparative pressor effects of a dose of 0·01 g./kg. of (5) paramethoxy-, (6) dimethoxy- and (7) trimethoxyphenyl-methoxy-ethylamines.

60 mm. rise by the same dose of compound (5) in the same animal. In regard to pressure action on the spinal cat compound (1) is therefore roughly 2½ times as powerful as compound (5).

The relative pressor activities of the same dose of compounds (5), (6) and (7) on the same spinal cat are shown in Fig. 4. For this figure the tracings were separately photographed to the same scale and a single print taken through three negatives by the method described in the previous paper. It shows, as was found in other experiments, that the pressor effect of (5) is much greater than that of (6) or (7).

Fig. 5. Records as in Fig. 2. Rabbit anæsthetized by urethane. Effects produced by *p*-methoxyphenyl-methoxyethylamine, 0·01 g./kg. intravenously.

(*b*) *Rabbit.* In the anæsthetized rabbit compound (5) produced in a dose of 0·004 g./kg. a rise of blood-pressure of about 20 mm. Hg; 0·01 g. a fall of the same amount (Fig. 5), and 0·02 g. proved fatal from respiratory and cardiac failure. The other two compounds produced negligible effects on blood-pressure, even in doses of 0·01 g./kg.

Blood-pressure experiments in rabbits and cats do not suggest a sympathomimetic action, if one excepts the effect of compound (5) on the

decerebrate cat. The absence of other evidences of peripheral sympathetic stimulation suggests some other explanation of this pressor effect, the most probable being a nicotine-like effect which has been postulated for some other related amines. A point of interest is the difference in the pressor effect in the spinal, as opposed to the anæsthetized intact cat, in the case of compound (5). The pressor effect is much enhanced in the former case. A similar condition of affairs has been found with several alkaloids and amines investigated in this laboratory. The most obvious explanation, that in the spinal animal the possibility of reflex vagal stimulation is cut out and therefore the pressure rises higher, is not

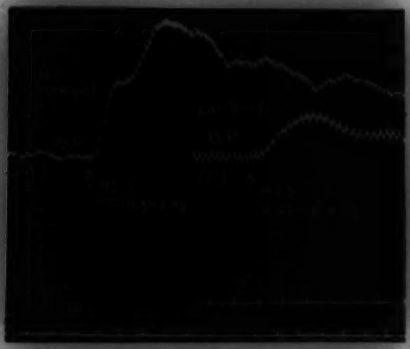


Fig. 6. Pressor effects of *p*-methoxyphenyl-methoxyethylamine, 0·004 g./kg. (*A*) in a spinal cat, (*B*) in an anæsthetized intact cat.

sufficient to explain all the cases, though it may be sufficient to explain the similar phenomenon in the case of adrenaline itself. An example of this difference is shown in Fig. 6 where the same dose of (5) was given (*A*) to a decapitate cat and (*B*) to an anæsthetized intact cat. The difference in the pressor effect is striking. (Tracing *B* was superimposed upon tracing *A*.) The difference is even more marked with larger doses, because whereas 0·01 g./kg. produced a fall of blood-pressure of an anæsthetized cat (Fig. 2) the same dose in a decapitate cat (Fig. 4) produces a rise of 126 mm. Hg. Though the pressor effect in the decapitate animal may be due to a nicotine-like stimulation of the ganglia in the course of the vaso-constrictor nerves, it is difficult to imagine that this stimulation could be so completely prevented by anæsthesia alone. We propose to study this

point further in the case of an alkaloid of which a greater supply is available.

Additional evidence as to the presence or absence of sympathomimetic action was obtained from experiments on the uterus and intestine of rabbits and cats. The organs were tested both *in situ* and exsected. In the former case the anæsthetized or decapitate animal was immersed up to the thorax in a saline bath at body temperature; the abdomen was opened and the uterus at some point near the vaginal end was attached to a rigid rod, and a hook, inserted in the uterus nearer by an inch or more to the ovarian end, was connected by a thread to a lever which recorded the uterine movements. A similar method was used for recording intestinal movements.

Fig. 7. Movements of isolated uterus of non-pregnant cat. Effect of 1 in 50,000 of (1953) *p*-methoxyphenyl-, (1954) dimethoxyphenyl- and (1955) trimethoxyphenyl-methoxyethylamines.

Uterus. Cat. The non-pregnant cat's uterus is inhibited by adrenaline and is therefore a convenient tissue for differentiating between a sympathomimetic action and a direct stimulant action on uterine muscle. All three compounds stimulated the non-pregnant cat's uterus whether isolated or *in situ*. This is shown in Figs. 2 and 3. In one experiment in a spinal cat, 0·001 g./kg. of compound (5) produced a rise of tone and augmentation of contractions while a subsequent equal dose of compound (1) produced a fall of tone and diminution of contractions. In another similar experiment compound (5) produced contraction and adrenaline relaxation of the uterus *in situ*. Fig. 7 shows the effect of 1 in 50,000 of compounds (5), (6) and (7) on the isolated uterus. The uterus is stimulated in each case, compound (6) being the least active.

So far as the cat's uterus is concerned, therefore, none of these compounds shows a sympathomimetic action. The stimulant action is due to a direct action on the uterine muscle.

Rabbit. The rabbit's uterus was likewise stimulated by each of the compounds. This is well shown by the intact animal for compound (5) in Fig. 5. On the isolated rabbit's uterus compound (5) was most active, compound (7) least. On the isolated cat's uterus compound (5) was most active, and compound (6) least.

Intestine. On the isolated intestine of both rabbit and cat all three compounds have a stimulating effect. The intestinal muscle is less sensitive than the uterine muscle to all of them; for example, a solution of 1 in 100,000 of compound (5) produced quite a pronounced contraction of the uterus, while 1 in 25,000 was the lowest concentration to act on the intestine.

In one experiment compounds (1) and (5) were compared on the same piece of cat's intestine, both in concentrations of 1 in 25,000; the former produced relaxation and the latter a rise of tone. On the intestine *in situ*, all compounds invariably produced an augmentation of movements in the rabbit (Fig. 5). In the cat, however, the results were variable. In some animals all four had a pure inhibitory effect (as in Fig. 3), in others the effect was motor, in others a motor followed by an inhibitory effect (as in Fig. 2).

Discussion.

The object of the present paper was to determine the effect of substitution of —H by —OCH_3 in the β position of the ethylamine side-chain on monomethy-, dimethoxy- and trimethoxyphenylethylamines. It had been shown in a previous paper that *p*- and *m*-methoxyphenylethylamine had a sympathomimetic action in the cat but not in rodents and a stimulant action on the central nervous system; whereas dimethoxyphenylethylamine had no sympathomimetic action even in cats and had a purely depressant action on the central nervous system, in both these respects resembling trimethoxyphenylethylamine (mescaline). The presence of one nuclear methoxyl group does not entirely remove the sympathomimetic action which is still present in cats; the presence of two or three such groups removes this action entirely. It was hardly likely, therefore, that the addition of a methoxyl group to the side-chain of compounds already containing two or three nuclear methoxyl groups would restore the sympathomimetic action, or the stimulant action of the central nervous system. In accordance with anticipation it has been found that the addition of a methoxyl group to the side-chain of dimethoxy- and trimethoxyphenylethylamine merely weakens their physiological activity without apparently altering their actions qualita-

tively. With this addition both substances remain depressant to the central nervous system and devoid of peripheral sympathomimetic action.

Where it was less easy to make any confident prediction was as to the effect of a methoxyl group on the side-chain (5) of (1) monomethoxyphenylethylamine. Would the change abolish the stimulant action on the central nervous system and the sympathomimetic action in cats? How would the resulting compound compare with (2) dimethoxyphenylethylamine with which it is isomeric, though one of the methoxyl groups is differently attached in the two compounds?

So far as the central nervous system (in mice) is concerned, the stimulant action is marked with (1), feebly present in (5), and absent in (2). In regard to sympathomimetic action the following points are of importance. Compound (5) has no augmentor-accelerator effect on the exsected hearts of frogs, rabbits or cats. It does not inhibit the movements of the isolated non-pregnant cat's uterus or of the isolated intestine. In all these respects it differs from compound (1) and fails to reveal any peripheral sympathomimetic action of the type of adrenaline. Two of its effects might be interpreted as due to such an action, viz. pressor effect and the variable inhibition of the intestine *in situ* in the cat. Those effects are, however, capable of other explanations: *e.g.* an action on sympathetic ganglia of the type of nicotine or (as suggested by Raymond-Hamet for dimethoxyphenylethylamine) of sparteine.

Summary.

The physiological actions of (5) *p*-methoxyphenyl-β-methoxyethylamine, (6) 3-4 dimethoxyphenyl-β-methoxyethylamine, and (7) 3:4:5 trimethoxyphenyl-β-methoxyethylamine have been investigated and compared with the corresponding compounds lacking the methoxyl group on the side-chain; the actions of the latter were described in a previous paper.

The approximate minimum lethal dose by intraperitoneal injection for mice was found to be, in g./kg., (5) 0·35, (6) 0·5, (7) 0·5.

The addition of a methoxyl group to the side-chain diminishes physiological activity. Compounds (6) and (7) have a depressant action on the central nervous system and no peripheral sympathomimetic action; in both respects resembling the corresponding compounds without the side-chain methoxyl group.

Compound (5) differs from *p*-methoxyphenylethylamine as follows: it possesses about two-fifths of the toxicity for mice and of the pressor

action in decapitate cats; it has lost the sympathomimetic action which the latter compound exhibits in cats; it has a weaker stimulant action on the central nervous system.

All three compounds stimulate smooth muscle, of the intestine and uterus.

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EFFECTS OF RESPIRATION UPON THE VENÆ CAVÆ OF CERTAIN MAMMALS, AS STUDIED BY MEANS OF X-RAY CINEMATOGRAPHY.

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(Received March 29, 1934.)

The problem indicated in the title was suggested by the previous work of one of us (K. J. F.), who was also responsible for the operative procedure. The solution of the problem was made possible by the use of X-ray cinematography, the technique of which has been largely developed, since 1926, by the other of us (R. J.).

The effects of respiration upon the calibre and volume of the thoracic inferior vena cava of the cat, dog, and rabbit have already been estimated by an indirect method (K. J. F.), and the findings corroborated, in the case of the cat, by X-ray cinematography (R. J.) [Franklin, 1933]. Although it is incorrect to draw too general a conclusion from so small a number of experiments, it seems probable that the volume of this vein in these three species is diminished rather than increased in inspiration, especially if inspiration is at all deep. It is of interest to note that Eyster and Hicks [1933], on other grounds, came to the belief that in dogs "the importance of breathing in reference to venous return has been exaggerated in the usual theoretical discussions of this subject."

The thoracic inferior vena cava may also be constricted to some extent at its diaphragmatic end in certain species (*e.g.* cat and dog) when inspiration is at all deep. This effect is not to be regarded as of outstanding importance, for it depends primarily upon the degree of development of a particular anatomical feature, and the animals examined showed considerable variation in this respect [Franklin, 1934]. In the present series of experiments an inspiratory constriction of the diaphragmatic end of the thoracic inferior vena cava was clearly visible on the X-ray screen

in the case of the second dog (Plate I, fig. 2). The post-mortem examination showed that this animal had a moderately well-developed "caval band," and this was almost certainly responsible for the constriction.

The experiments described below were undertaken in order to add to the knowledge already acquired of the effects of respiration upon the calibre and volume of the venæ cavæ. The particular portions of the caval system about which further information was required were the superior vena cava, and the intrahepatic portion of the inferior vena cava. For the greater part of this work there was only one suitable technique, namely, X-ray cinematography, and this technique was therefore employed.

It is perhaps important to emphasize the fact that the rate of flow of blood in the venæ cavæ was not primarily under consideration; a study of the X-ray film of experimental pulmonary embolism made by Naegeli and Janker at Bonn [Naegeli, 1931] shows that the movement of fluid within the venæ cavæ is a complicated one, and we have, therefore, been content to study chiefly the changes in calibre and kindred problems.

Method.

The technique of X-ray cinematography, as applied to animals, has been adequately described elsewhere [Janker, 1932 and 1933]. It is only necessary, therefore, to state that we employed the indirect method, *i.e.* that we took cinematograph pictures of the X-ray screen upon 35 mm. film at the standard rate of 16 per sec. The screen lay in all cases immediately below the animal, and some distance above the cinematographic recording apparatus.

Three cats, three rabbits, and three dogs were used for the experiments. During the whole of the time they were under ether anæsthesia, and they were killed immediately after the photographs had been secured.

From these nine animals films were obtained showing the passage along the superior vena cava and the inferior vena cava of a solution opaque to X-rays. The solution used was 50 p.c. sodium iodide. Photography was carried out with the animals in the supine and in the lateral positions.

A solution of sodium iodide of the concentration indicated may of itself [Schade and Wohlleben, 1933] cause some degree of contraction of muscular veins. It is possible, therefore, that our results show only the qualitative, and not the quantitative, effects of respiration upon the venæ cavæ. But against this it may be pointed out that the contrast

solution was in all cases progressively diluted by the blood returning from the opposite side of the body, and by blood coming from tributary veins *en route*.

The injections were made into the femoral vein for the inferior vena cava, and into the external jugular vein for the superior vena cava. The fluid was injected under a minimal pressure from a large syringe, which was connected to the vein by a length of rubber tubing and a venous cannula. It is difficult to estimate how far this procedure disturbed the normal conditions of the venous return, but it is possible that the injection more or less replaced the return through the interrupted femoral or jugular vein. The recording required only a matter of seconds in each case.

The strong sodium iodide solution slowed the heart, especially in the rabbits, but usually not before two or three normal respirations had been photographed. In any case, a failing right heart is at once demonstrated on the X-ray screen by a regurgitation of the contrast solution into the hepatic veins [Naegeli and Janker, 1931] and, in the experiments here recorded, the injections were stopped immediately any such regurgitation was observed.

Only one thing more needs mention here, and that is that one cannot say, with scientific certainty, that the contrast solution is everywhere in contact with the vein wall. The objection is, however, largely a theoretical one and, after observing a considerable number of injections on the X-ray screen, one cannot help concluding that the injected fluid is well and speedily mixed with the blood coming into the vena cava from the opposite side of the body. While one must, therefore, state the possibility, one may well believe that it is not a probability.

The condition of the animals, then, was as normal as we could make it during the actual recording, and the results can be accepted without too much reservation. The chief drawback, from our point of view, was that the ether anæsthesia tended to reduce the depth of respiration; the findings to be described would probably be greater in degree in the normal animal, especially if it were in active movement.

Results.

The effects of the respiratory movements upon the venæ cavæ have been studied in four ways. First, we observed the fluorescent screen during the course of the injections; the human eye cannot see sixteen pictures a second, but the screen picture is clearer than the photographic record taken of it; we therefore formed some valuable preliminary

observations in this way. Secondly, we have projected the film several times and at different rates; we have also reversed it in places and run portions of it through several times to convince ourselves of certain appearances. Thirdly, we have gone through the whole of the film, picking out parts where the respiration was good and the injection gave a satisfactory contrast; we have then projected these individual pictures as enlarged stills (23 × 21 cm.), and have drawn the outlines of the essential parts. The disadvantage of this procedure is that one cannot reproduce the depth of shadow shown by the injection. So, fourthly, to complete the examination, a certain number of photographic enlargements have been made of individual pictures.

Inferior vena cava. As a result of these four procedures, we are in a position to say that the effect of respiration upon the inferior vena cava in the species examined tends to be as follows. The thoracic inferior vena cava is somewhat narrowed in inspiration, and the amount of fluid at any particular point is decreased, as judged by the depth of shadow. (The total volume of the thoracic inferior vena cava could not be estimated, as the whole of this vessel was not included in the photographic records.) There is a varying degree of constriction of the vena cava in inspiration at the point where it emerges from the diaphragm. This occurs both in the dogs and cats, which, as has been shown, have a "caval band" of the diaphragm [Franklin, 1934], and also in the rabbits, where the action of the diaphragm is exerted in a slightly different way [*ibid.*]. The intrahepatic vena cava is compressed somewhat in inspiration and may, in certain cases, form a slight angle with the posthepatic portion of the vein. There are suggestions in the film in two or three places, where a small portion of injection fluid remains in the intrahepatic vena cava, that this is forced backwards a little in inspiration. We use the word "suggestions" because the effect is more clearly seen by the second procedure mentioned above than it is by the third; the dynamic action is not so clear in the enlarged stills, owing to the difficulty of defining the exact outlines of the shadows.

There are some exceptions to the above general picture, but we are of the opinion that they would have disappeared had inspiration been deeper. For instance, the thoracic inferior vena cava did not become narrower, but slightly wider, in one dog just at the beginning of inspiration. A similar result was also found by one of us (K. J. F.) in the case of one dog's isolated thoracic inferior vena cava in 1933; the same vein thereafter decreased in volume with greater lengthening until at a normal inspiratory length and pressure it had a smaller volume than at expiratory length and pressure.

The plate accompanying the present paper shows all the typical effects except the bending of the vena cava upon itself near its entry into the caudal end of the liver. In this plate the thoracic inferior vena cava is slightly narrower in inspiration (as measured in enlarged stills); it is also emptier (as shown by the smaller depth of shadow); the vena cava is constricted at its exit from the diaphragm, presumably through the action of the "caval band," and the intrahepatic vena cava is narrower than it is in expiration.

It must be pointed out that the auricular contraction sends a pulse wave backwards along the thoracic inferior vena cava, and it is possible that some of the exceptions to the general effects detailed above are due to this; unfortunately, the whole of the heart could not be included in the pictures, and it was impossible, therefore, to take the effect of this factor into consideration in any particular case.

Superior vena cava. Owing to a rapid to and fro movement of the greater part of this vein in the dog, it was impossible to make outline drawings of it in enlarged stills. In the rabbit the vein is very short, but there was a tendency for it to increase very slightly in calibre in inspiration. The increase was too small to be visible when the film was projected, and even in the enlarged stills it was minute. In the cat the vein is longer and the heart moves caudally for a certain distance in inspiration. As a result of our examination of a number of drawings of enlarged stills, we can state that there is a tendency for this vein to increase a little in volume in inspiration. Here again, however, the increase is too small to be visible when the film is projected in motion.

Discussion.

The results tend to confirm Haller's findings, mentioned in a previous paper [Franklin, 1933], that in these species the effect of inspiration is to facilitate inflow of blood from the superior vena cava, and to impede somewhat the inflow of blood from the inferior vena cava. We should like to point out that the conditions in man are different from those found in these lower animals, and that, although respiration may be found to act upon the venous return in man to a similar end by a slightly different mechanism, it cannot be assumed to do so without more definite experimental proof.

Further work is being done to show the effect of respiration upon the posthepatic vena cava, and the results so far obtained support the views described above.

Fig. 1.

Fig. 2.

Figs. 1 and 2. Lateral views, taken from the X-ray film, of part of the inferior vena cava of a dog during an expiration (above) and the inspiration immediately following it (below). The vena cava contains X-ray opaque solution (sodium iodide). *T.I.V.C.* thoracic inferior vena cava; *D.* rostral edge of the diaphragm shadow; *I.V.C.* intrahepatic vena cava.

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Conclusions.

In the species studied (dog, cat and rabbit) there is, during inspiration, a tendency for the inferior vena cava to be constricted at its exit from the diaphragm, and for the intrahepatic vena cava to be compressed and narrowed. At the same time the superior vena cava tends to widen very slightly, or to increase in volume; this possibly holds true for all three species mentioned, but for the reasons stated above it can only at present be regarded as proven in the cases of the cat and of the rabbit.

We wish to thank Prof. Frhr. v. Redwitz for the facilities afforded us in the University Surgical Clinic, Bonn, and Prof. Naegeli for his interest and cooperation. For help in the conduct of the experiments we are indebted to Frl. Kaas and Frl. Sanders, X-ray assistants, and to Herr Bötsch, technician. The expenses of the research were defrayed by a special grant made to one of us (K. J. F.) by the Rockefeller Foundation, and we wish here to record our appreciation of the Foundation's generous action. The work forms part of a study of the venous return in mammals, and the film was shown to the Physiological Society at its meeting on February 17, 1934.

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THE INTERPRETATION OF POTENTIAL WAVES IN THE CORTEX.

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(*Received April* 4, 1934.)

If two electrodes are placed on the surface of the brain a record of the potential difference between them shows repeated fluctuations which may be as large as a millivolt. Recent work on this subject dates from the string galvanometer investigations of Prawdicz-Neminsky [1913, 1925], and in the last five years a number of papers have been published from various laboratories. A review of the literature prior to 1932 has been given by Fischer [1932]. It has been shown that the potential waves are generated in the cortex, that they are modified by stimulation of sense organs and abolished by cutting off the oxygen supply to the brain; in fact there is no doubt that they are due to the activity of the cortical neurones. Recording them would seem to offer the most direct method of investigating cortical activity, but for the difficulty that they are certainly summated effects compounded out of the potential changes in many neurones. It is most unlikely that the change in each neurone is an exact copy, on a smaller scale, of the massed effect, and we cannot go much further until we know how the massed effect is built up.

The problem, then, is to decide what kind of changes to expect in the individual neurones. The potential waves recorded in the cortex are usually irregular and show wide variations in size and duration. Are the action potentials of the cortical cells as variable, or are they as uniform as the impulses in a nerve fibre? If the potential between two points on the brain increases for half a second and then decreases, are we to suppose that potential gradients have developed equally gradually in certain neurones, or that the effect is due to repeated brief outbursts which increase in number as more and more units are involved and as their activity rises? The question has been raised before by one of us [Adrian, 1931] in connection with the slow potential changes in the nerve ganglia

of insects. It was suggested that the depolarization of a nerve cell might be a much more gradual process than the explosive change which occurs in the nerve fibre. In the present work, however, we have found no evidence in favour of very slow changes in the individual neurones, and we have reached the conclusion that the cortical effect is mainly built up out of repeated brief pulsations, slower than the potential waves in a nerve fibre but of the same general character.

Technique.

All our experiments have been made on animals under a general anæsthetic. The bearing of this on the results will be discussed later; for the present it is enough to say that the anæsthetized brain shows considerable electrical activity and that other workers have found no evidence of a radical change in the character of this activity when consciousness is lost. A few experiments were made on cats; the majority on rabbits under chloroform and ether, or urethane. Chloralose, dial or pernocton were used occasionally.

The brain was exposed by trephining the skull over the parietal region and enlarging the opening to about 2×2 cm. Bleeding from the bone was controlled by plasticine, the dura was opened and turned back, and the surface of the brain was repeatedly irrigated with warm Ringer. The head was held in a clamp but the body was not secured in any way. In some experiments respiration was recorded by a tambour connected to a branch from a tracheal cannula and the electrocardiogram by leads on the head and in the anus.

For leading from the surface of the cortex the electrodes were usually of the non-polarizable type in which a coil of silver wire coated with $AgCl$ dips into a glass tube containing Ringer's fluid. For many experiments we used tubes plugged at the lower end with china clay through which a worsted thread protruded to make contact with the cortex. For leading from very small areas with electrodes close together the tubes were drawn out to a point with an external diameter of 1 mm. or less and plugged with gelatin, the orifice of the tube resting lightly on the cortex.

The concentric needle system [Adrian and Bronk, 1929] gives the most restricted interpolar field, but we have found it troublesome to use on the brain. If the outer tube and inner wire are both of silver the area which can be coated with $AgCl$ is not large enough; the electrodes often develop a high resistance after they have been in contact with the brain for 10–20 min. If the tube and wire are not of the same metal, local circuits are often set up and may produce troublesome artefacts. Pointed gelatin electrodes are more reliable, though we have not tried to develop a concentric type.

For leading from the deeper layers of the cortex we have used both the pointed gelatin type and the concentric needle system (of silver), but many records have been made with a large indifferent electrode on the surface and an enamelled silver wire, bared at the tip and coated with $AgCl$. This could be thrust through the cortex with a minimum of damage.

The methods we have used for analysing the cortical waves have depended mainly on the comparison of simultaneous records from three

pairs of electrodes with three independent amplifying systems and three oscillographs. To begin with the amplifiers were of the usual type, one of the input leads being connected with the grid of the first valve and the other to earth. Thus one of each pair of leads was earthed, and as nothing was to be gained by using three distinct earthed electrodes we used a common one of large area on the cortex or in the mouth, with separate electrodes to the three grids. It was soon obvious that such an arrangement gave unsatisfactory results. The brain is a large mass of tissue in which all manner of potential changes are taking place; when the grid electrode rests on a point on its surface and the other is earthed and forms a diffuse lead from the whole mass, there is no reason to suppose that a change in their relative potentials is due to changes occurring at the point where the grid electrode is applied. The brain at this point may be inactive and the average potential of the rest of the brain (relative to the point) may have altered. With three pairs of electrodes, one of each pair going to a common earth, or with three grid electrodes and a single diffuse earthed lead the position is much the same—the simultaneous appearance of a potential wave at the three pairs does not necessarily imply simultaneous activity at the three regions to which they are applied. For this reason it is exceedingly difficult to draw any certain conclusions from the records. The difficulties which arise in this way are illustrated by a control experiment made on the frog with three concentric needle electrodes. The outer tube of each needle was earthed, the inner wires leading to the grids of the three amplifiers. With this arrangement if one needle system was on the heart and the other two on the liver, all three showed potential waves corresponding to the electrocardiogram. A single needle system on the liver showed only a minute disturbance, but connecting the outer tube with electrodes in other parts of the body caused its potential to fluctuate relative to that of the inner wire and so brought back the wave at each heart beat.

To overcome the difficulties introduced by the common earthed lead we adopted an amplifying system in which neither input lead was earthed. The system [Matthews, 1934] has been described elsewhere in detail, as it has some additional advantages over the usual form. Two input valves are used in a balanced circuit, the two electrodes lead to the two grids and the resulting changes in anode current are handed on through condensers to the later stages of the amplifier, which are of the usual condenser coupled type. With this arrangement the three pairs of electrodes are quite independent; a pair on the frog's liver shows only a very small deflection, although other pairs on the heart give simultaneous records

of the electrocardiogram (Fig. 1). Three Matthews' oscillographs were used to record the potential changes, which were photographed and could be viewed with the usual rotating mirror arrangement. A loud-speaker was not often used, as the cortical potentials often rise and subside too slowly to give clearly audible sounds. Our records of the cortical potentials are sometimes slightly distorted by the use of capacity-coupled amplifiers, but this has been controlled by the use of a battery-coupled amplifier (see p. 455) and the distortions are not of a kind which would affect the interpretation of the records.

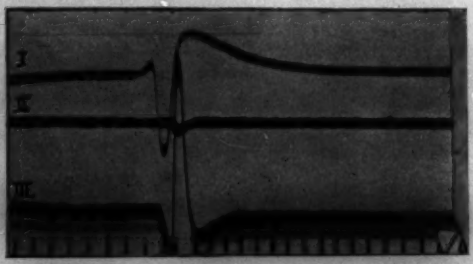

Fig. 1. Frog's electrocardiogram with balanced amplifiers. Three concentric needle electrodes, I and III on the heart, II on the liver. Sensitivity the same for all. Time marker gives 1/20 sec. With three earthed amplifiers the deflections from the liver are as large as those from the heart.

Results.

Characteristic potential changes from the cortex.

(*a*) *Rhythmic outbursts in deep anæsthesia.* In an animal which is lightly anæsthetized and still more in one which is not under a general anæsthetic [*vide* Fischer, 1932, 1933; Kornmüller, 1933; Tönnies, 1933] the potential changes in the cortex are irregular and may be difficult to predict. Under deep anæsthesia they become much more regular, and although there are variations from one rabbit to another we can make fairly certain of recording the same type of disturbance again and again over periods of several hours. We had not expected this result, though it is mentioned by Bartley and Bishop [1933]. It has been of great value, for it has enabled us to plan experiments knowing what kind of cortical response to expect.

Fig. 2 illustrates, in A, the type of record usually found in light urethane anæsthesia, and in B and C, the more regular type with deeper anæsthesia. In the latter a series of outbursts occur at intervals of 1–3 sec. Each consists of one or more large elevations rising and declining relatively slowly with a series of much smaller and briefer oscillations superimposed on them. The slow elevations represent potential changes of 0·5–1·0 millivolt. If the amplification is of the order needed to keep the whole of these waves on the record there is usually no sign of activity in the intervals between each outburst, but with higher amplification it is possible to detect oscillations of the brief type scattered through most of the quiet interval.

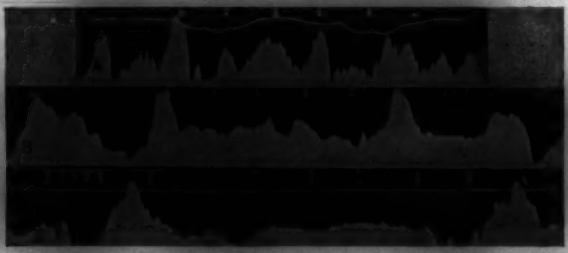

Fig. 2. Potential changes from cortex of rabbits under urethane anæsthesia. A, moderately light; B and C, deep. In deep anæsthesia the slow waves become more regular. The "brief" waves are just visible. Condenser-coupled amplifier, non-polarizable thread electrodes 1 cm. apart on cortex. White line in record A signals respiration. Time marker gives 1/4 sec. intervals.

Under urethane the outbursts seldom occur with complete regularity; more definite rhythms appear in rabbits under chloroform and ether. Those in Fig. 3 are typical of moderate degrees of anæsthesia (corneal reflex just abolished). The slow waves have a characteristic frequency of 3–4 per sec. with brief waves at 25–40 per sec. superimposed. Simultaneous records of the electrocardiogram and of respiration show that the cortical rhythm is quite independent, although these extraneous rhythms are often of the same order. In very deep anæsthesia (Fig. 4), the 3–4 per sec. rhythm gives way to a slower beat at intervals of 1–2 sec. The superimposed brief waves are on the whole more regular, and their frequency usually lies in the region of 25–30 per sec.

Both slow and brief waves are due to the activity of the cortex. It is true that when a large earthed electrode is used they persist after the

cortex under the grid electrode has been killed by freezing with solid CO_2, but this is due to the dead tissue acting as a lead from neighbouring

Fig. 3. Rhythmic waves from the rabbit's cortex in moderate C. and E. anæsthesia. Four different animals. Slow waves at 3–4 per sec. with brief waves superimposed. Respiration shown in B and C and electrocardiogram in D (upper line), not in phase with the slow waves. In D there are two records from different regions but the rhythm only appears in the lower. Time marker in A, B and C gives 1/4 sec. In D horizontal line = 1 sec.

Fig. 4. Slower rhythm in deep C. and E. anæsthesia. A and B from one rabbit; A, moderate anæsthesia, waves 3·5 per sec.; B, deep, waves 1 per sec. C and D from another rabbit; C, moderate anæsthesia, waves 4 per sec.; D, deep, waves 1·3 per sec. Respiration not in phase.

regions, for with the balanced amplifier two electrodes on the killed area record little or nothing. Subcortical structures may possibly contribute to the effect under certain conditions, but we have no evidence as to this.

(*b*) *The injury response.* Another characteristic type of activity is that produced by injury to the cortex. It can be produced without fail, and it supplies important evidence as to the nature of the potential waves. If two electrodes rest at some distance apart on the cortex and a small cut is made in its surface near one of them, there is usually an outburst of brief potential waves in a regular series with a frequency somewhere between 90 and 35 per sec. A cut 1 mm. long and ½ mm. deep is often enough, but a puncture with a fine wire to the same depth is usually without effect. On the other hand if a wire, a pointed gelatin electrode, or a concentric needle system is gradually pushed through the superficial layers of the cortex there is nearly always an outburst of the regular waves when the point has penetrated to a depth of 1·5–3 mm.

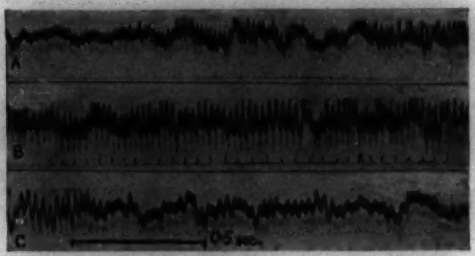

Fig. 5. Evolution of a typical "injury discharge." Rabbit under urethane. Puncture of cortex to 2–5 mm. depth by a fine silver wire forming the input electrode. A, beginning of discharge, frequency 64 per sec.; B, middle, frequency 44 per sec.; C, end, frequency about 40 per sec. during regular periods.

These injury discharges are clearly due to structures in the grey matter. In the rabbit's brain this has a thickness of about 2·5 mm., but the response of the white matter can be readily distinguished, for at a depth of about 3 mm. the response changes to a succession of small and very rapid oscillations like those given by a large nerve trunk pierced by a needle electrode. They are evidently due to impulse potentials in the axons, whereas the injury discharge is due to nerve cells and dendrites[1].

The response of the grey matter, whether to superficial or deep injury, shows a characteristic evolution as well as a characteristic frequency

[1] As there is no term which covers both the nerve cell and its dendrites but excludes the axon, we have spoken of the "cortical neurones" or "cortical nerve cells" as responsible for the potential waves. Some nervous constituent of the grey matter is responsible for them, cell bodies or dendrites or both.

range. The waves often begin a few seconds after the injury. They are small at first and can sometimes be seen as discrete, monophasic spikes with a duration of about 5–10σ. They become larger, and as they do so their outline broadens until the record becomes a sinusoidal oscillation. Usually the frequency reaches the maximum value (60–80 per sec.) at once and then falls gradually to a value in the region of 50 per sec. Occasionally the initial frequency is about 40–50 per sec. and the maximum is not reached for several seconds. The discharge may continue at about 50 per sec. for as long as half a minute, but eventually the frequency falls to 35–40 per sec., the waves begin to vary in size and the regularity ceases (Fig. 5). With more widespread damage the response usually appears after a shorter interval: the initial frequency may be as high as

Fig. 6. A, beginning of injury discharge (rabbit, urethane). Waves alternately large and small, frequency 90 per sec. B, beginning of injury discharge (rabbit, urethane). Monophasic waves, frequency 40 per sec. (increased later).

100 per sec., and in the later stages occasional very large waves may occur, often diphasic. In many discharges there is a stage in which the successive waves are alternately large and small (Fig. 6), and sometimes the final break up of the rhythm is heralded by an alternating rhythm of this kind. Injury discharges have been recorded in twenty or more rabbits, and the frequency limits have shown no significant variation. The response to a superficial injury can be given a faster or slower rhythm by irrigating the surface of the cortex with hot or cold Ringer. The depth of anæsthesia has no obvious effect on the frequency, but in very deep C. and E. anæsthesia a greater injury may be necessary to produce the discharge. More severe injury seems to be needed in the cat than in the rabbit, though the discharge has the same frequency range and the same evolution (Fig. 7).

The characteristic evolution suggests that we are dealing with a synchronous pulsation in a number of units. Synchronous discharges

due to injury have been recorded in nerve [Adrian, 1930; Hoagland, 1933] and in muscle [Adrian and Gelfan, 1933], and in these structures, as in the cortex, the response consists of large regular oscillations which approach a sine curve but become small and less regular as soon as the frequency falls below a critical value. In the cortex it appears that the activity is at first confined to a few neurones and then gradually spreads to include more and more. This would account for the increase in size and the broader contour of the waves. Direct evidence of such a spread can often be obtained by recording simultaneously from two or three pairs of

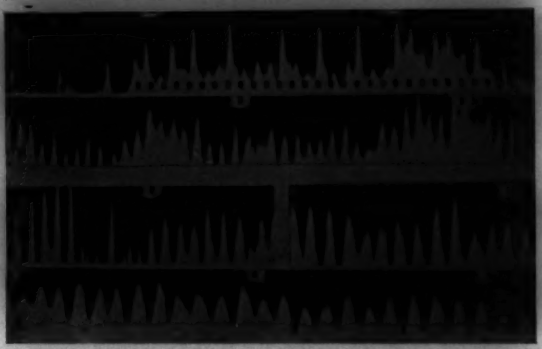

Fig. 7. Evolution of injury discharge in cat (C. and E. anæsthesia). Puncture of cortex by fine wire. A, large monophasic waves 30 per sec.; B, later, frequency 76 per sec.; C, later, frequency 72 per sec.; D, later, frequency 56 per sec.; E, later, frequency 48 per sec. Time marker gives 1/4 sec. intervals.

electrodes. Fig. 8 gives two examples of this. Further evidence of the synchronous pulsation comes from the fact that two electrodes placed very close together near the injured point often fail to show any sign of activity, whereas either of them in conjunction with a distant electrode shows large oscillations. Clearly no potential differences can be developed between two electrodes if both of them are on an area whose potential rises and falls synchronously at every point, but the oscillation will appear if one of the electrodes is removed to an inactive or less active region.

The units responsible for the injury discharge must have slower time relations than those of medullated nerve fibre. In a mammalian nerve trunk synchronous activity can occur over a frequency range of 600–120 per sec. In the cortex the range is about 100–35 per sec. Rhythms as high

as 200–300 per sec. have been observed occasionally with stimulation by a constant current, but 80–35 per sec. is the characteristic range. The form of the waves is another argument for slower time relations. The fully developed sinusoidal oscillation can only tell us that a number of units are pulsating together at a particular frequency, but there are often periods, sometimes at the beginning and sometimes towards the end of an outburst, when the waves are separated by distinct pauses (Figs. 6 B, 7 A).

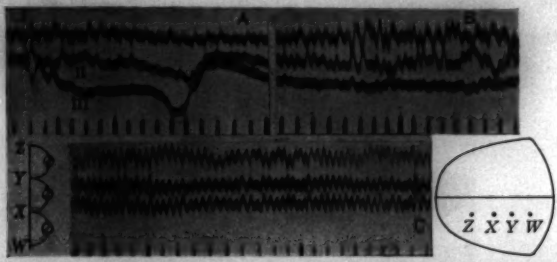

Fig. 8. Spread of injury discharge. Records from different points on the cortex (rabbit, urethane). A and B, successive stages in one experiment with four electrodes in line at intervals of 4, 3 and 3 mm. and three recording systems (cf. inset, full size). Injury at *Z* electrode: in A, record between *Z* and *Y* gives waves at 54 per sec. and that between *Y* and *X* gives half rhythm; in B, all three records give waves at about 45 per sec.; C, another injury giving waves at 50 per sec. between each pair of electrodes. Balanced amplifiers. Time marker gives 1/20 sec.

Since they still have a smooth contour and an over-all duration of about 5–10 σ it is unlikely that the response of each unit is much briefer than this.

The analysis of cortical potentials.

The nature of the injury response has been discussed at length because the waves which appear in it give a fair idea of the rapidity of the changes which can occur in excited nerve cells, and because they resemble very closely the brief oscillations which occur spontaneously. In the records of spontaneous activity already given (Figs. 2, 3, 4), the brief oscillations cannot be seen very clearly, but the size of the slow waves can be reduced by using smaller coupling condensers, and it is then possible to study the brief waves under higher amplification. Though they can always be observed if the amplification is great enough they are usually most prominent in moderately light C. and E. anæsthesia (Fig. 3 A). They are

certainly more variable than the waves of the injury discharge. Moderately regular waves at frequencies below 35 per sec. are often found, particularly in deep C. and E. anæsthesia, and there are occasional groups of frequencies as low as 10 per sec.; but regular oscillations at 35–50 per sec. are usually present in parts of the record, and it is often quite impossible to distinguish a regular train of spontaneous waves from the regular oscillation of an injury discharge (Fig. 9). It is true that no hard and fast line can be drawn between what we have called "brief" and "slow" waves, but there is no doubt that at the "brief" end of the scale the waves resemble those produced by injury to the cortex.

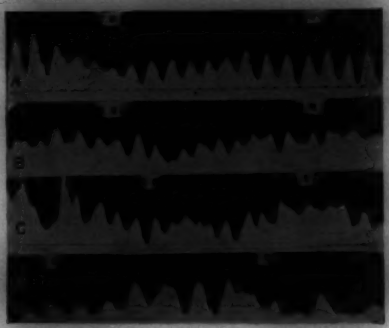

Fig. 9. Comparison of injury discharge and "brief waves" in spontaneous activity. Rabbit under C. and E. A, response to puncture of cortex: waves 48 per sec.; B, C and D, spontaneous activity in uninjured cortex with groups of brief waves; frequency between time signals, B 48 per sec., C 40 per sec. and D 36 per sec. (approximate). Time marker gives 1/4 sec.

Their likeness to the injury discharge shows that the brief waves represent the rapid pulsating activity of groups of nerve cells. Our problem, then, is to decide whether there is any other kind of activity. Are the slow waves merely summation effects compounded out of repeated brief pulsations in many neurones, or are some of the neurones capable of slow changes as well?

The fact that the brief waves in the injury discharge are initially monophasic has an important bearing on the question, for if the brief waves were invariably diphasic they could not summate to give a large, slow deflection. The waves which occur spontaneously are rarely separated by pauses distinct enough to show whether they are monophasic or

not, but at the beginning and sometimes at the end of an injury discharge there is no doubt of it. It might be argued that this is a natural consequence of injury, but the injury may be inflicted at some distance from the electrodes and the responses are still monophasic. Moreover, it would be natural to expect a monophasic potential change when a nerve cell becomes active, for one electrode may be regarded as leading from the point at which the activity originates and the other is a diffuse lead from

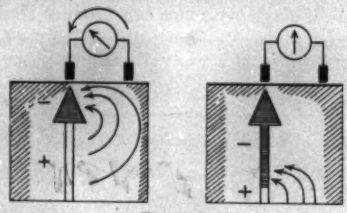

Fig. 10 A.

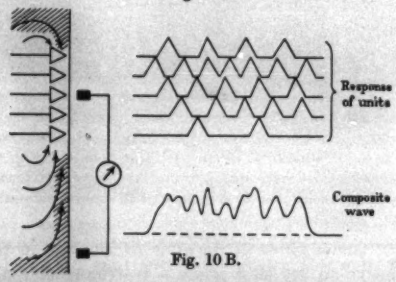

Fig. 10 B.

Fig. 10. A, potential gradients due to a wave of activity arising in a nerve cell and travelling down the axon, to show the production of monophasic waves: B, potential gradients due to repeated asynchronous activity in a group of nerve cells, to show the formation of the slow potential waves. For simplicity it is assumed that all the nerve cells will have the same effect on the recording system.

the whole of the axon (Fig. 10 A). Thus a series of rapid monophasic pulsations in a large number of neurones might give a composite potential wave in the way illustrated in Fig. 10 B. This would only occur if the pulsations were asynchronous, and it is noteworthy that in a synchronized injury discharge the slow waves disappear. This is perhaps the most direct evidence for the view that the slow waves are built up out of the same components as the injury discharge. The two types of activity are

mutually exclusive as they must be if one is due to asynchronous and the other to synchronous pulsations in the same neurones.

If the slow waves are in fact due to a summation of the brief waves they should be much more in evidence when the distance between the electrodes is large. If the distance could be made so small that the main effect came from a single neurone we should expect to find nothing but a succession of the brief waves, and the summation effect would become larger and larger as there were more and more neurones to contribute to it. On the other hand, if single neurones can produce slow as well as brief changes of potential these should be still evident when a very small electrode system is used.

Fig. 11. Change in relative sizes of "slow" and "brief" waves with change in separation between electrodes, Rabbit under C. and E. A, input electrode on cortex, earthed electrode in cheek. B, concentric needle electrode system 1 mm. diam. on cortex. Recording system 10 times more sensitive. The brief waves are much larger, but the slow are if anything smaller than in A in spite of the increased sensitivity.

All our experiments agree in showing an increase in the size of the brief waves relative to the slow when a restricted electrode system is used. Thus Fig. 11 compares the rhythmic waves in C. and E. anæsthesia as recorded (*a*) with a diffuse earthed electrode and one lead from the cortex and (*b*) with a concentric needle system resting on the surface of the cortex. The amplification in (*b*) is greater than in (*a*), so that the reduction in the size of the slow waves is much greater than appears in the record. Again, Fig. 12 shows the typical urethane outbursts recorded with three pairs of pointed gelatin electrodes arranged as shown in Fig. 12 A. In the upper records the sensitivity is the same for all three pairs. There is no sign of the slow waves at the pair of electrodes which are only 1 mm. apart, though the brief waves are not very much smaller than at the more widely separated electrodes. In the lowest record the sensitivity has been greatly increased for the 1 mm. electrodes: the brief waves

are more prominent but the slow waves are still greater at the other electrodes.

A similar result is reached when we use electrodes at different distances from the surface of the cortex. Tönnies [1933] has already compared the effect of leading from the cortex, the dura and the unopened skull, and shown the greater prominence of brief waves in the cortical record. We have used the arrangement shown in Fig. 13. A pool of warm Ringer was formed on the surface of the cortex by building a wall of dental cement

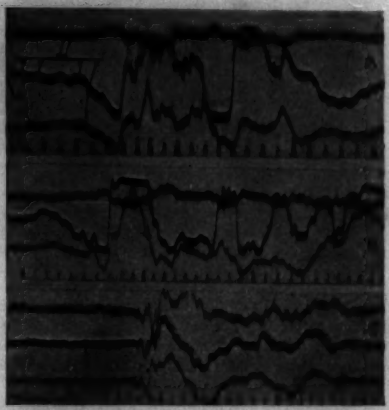

Fig. 12. Change in relative sizes of slow and brief waves with electrode separation. Rabbit under urethane. Balanced amplifiers. Electrodes arranged as shown in A; those to oscillograph I are 1 mm. apart, those to II 9 mm. and those to III 16 mm. In A and B all three oscillographs are equally sensitive. In C, No. 1 is made more sensitive (top record, 1 mm. between electrodes). Time marker gives 1/20 sec.

round the cut edges of the skull or by lifting up the scalp at each side. The electrodes were placed so that the points of one pair rested on the cortex with the others at various distances above it in the Ringer. The slow waves can be detected 6 mm. above the cortex, and in Fig. 13 B where the top pair of electrodes are 9 mm. apart and the bottom pair only 2 mm. there is no marked difference in their size, but in both records the brief waves appear only at the electrodes actually in contact with the cortex. Evidently the brief waves arise from structures very close to the surface electrodes, whereas the slow waves have a much wider or more distant origin. The diagram in Fig. 14 may make this clearer.

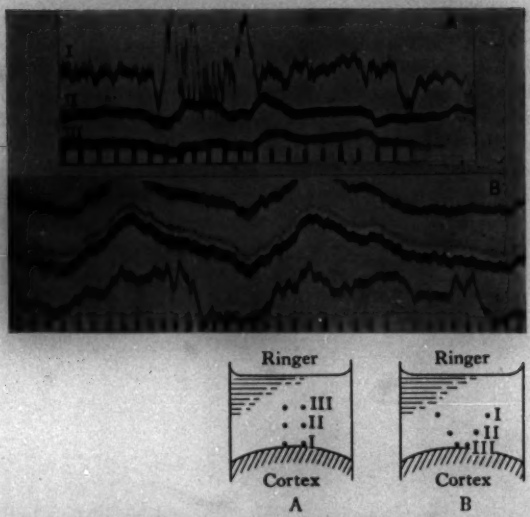

Fig. 13. Change in relative sizes of slow and brief waves with electrodes at different distances from surface of cortex. Pool of Ringer covering brain (rabbit, urethane). In A oscillograph I connected to electrodes 3 mm. apart on surface of cortex; II to electrodes 3 mm. apart and 3 mm. above surface; III ditto, 6 mm. above surface. I less sensitive —oscillographs adjusted to give approximately equal excursions.
In B, III is connected to surface electrodes 2 mm. apart; II to electrodes 4·5 mm. apart and 2 mm. above surface and I to electrodes 9 mm. apart and 5 mm. above surface. The very small oscillations in I and II are due to amplifier noise. Time marker gives 1/20 sec.

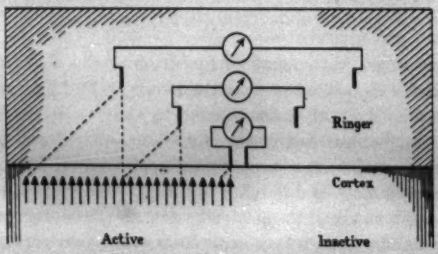

Fig. 14. Potential waves recorded by electrodes on the surface of the cortex and some distance above it (as in Fig. 13). The electrodes above the surface will record only the summated effect from a wide area. Those on the surface will be mainly affected by the neurones immediately under them.

Thus the composite nature of the slow waves is shown by our inability to localize their origin. They are greatest when a large mass of cortex intervenes between the two electrodes, but a detailed exploration of the mass, both in depth and on its surface, never reveals any region where the slowly developing potential gradients are greater than elsewhere. Their existence evidently depends on the activity of a large number of units, and the response of each unit bears no resemblance to them.

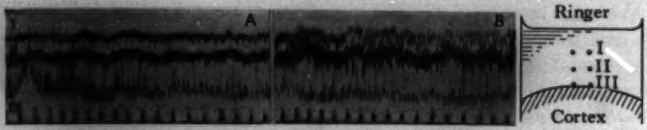

Fig. 15. Synchronized injury discharge from a large area appearing at electrodes 6 mm. above cortex. Rabbit, urethane. I, to electrodes 3 mm. apart and 6 mm. above surface (in Ringer); II, to electrodes 3 mm. apart and 3 mm. above surface; III, to electrodes 3 mm. apart and in contact with surface. In A the discharge is localized and has not much effect on the electrodes above the surface. In B it has spread and appears in all three recording systems. Time marker gives 1/20 sec.

Amplifier distortion.

As the foregoing experiments were all made with condenser-coupled amplifiers the slow waves will be distorted to some extent in the records. This does not affect the argument, but it is possible that the regions under the electrodes might sometimes change their potentials so slowly that nothing would appear in the record. To examine this possibility and generally to see how far our results are affected by amplifier distortion we have made some simultaneous records with a battery-coupled amplifier leading to one oscillograph and a condenser-coupled amplifier to another. With 1 mf. coupling condensers in the latter there is very little difference in the records, and in particular there is no indication of gradual (or sudden) shifting of the base line with the distortionless amplifier. Between the slow waves the potential returns to its original value just as it does with condenser coupling. Thus there is no evidence of very slow potential changes either in the cortex at large or, *a fortiori*, in the individual neurones.

Areas contributing to brief and slow waves. (*a*) *The brief waves. Synchronous pulsation of neurones.* The existence of the slow waves implies an asynchronous pulsation in the different parts of the area which contributes to the potential gradient, for if all the neurones were pulsating synchronously we should record a rapid oscillation instead of a slow rise or fall. The widespread synchronous pulsation caused by injury does in fact appear as such at electrodes as much as 6 mm. above the surface of the cortex (Fig. 15), but this distance is enough to smooth out all trace of the brief waves in the normal spontaneous activity. On the other hand, it is

unlikely that the brief waves are due to single nerve cells, for the magnitude of the potentials (which may be as much as 100 microvolts) and the sinusoidal wave form both suggest a synchronous pulsation in a group of cells.

We have attempted to find out how large an area contributes to each of the brief waves, for this will give some idea of the magnitude of the group which is pulsating together. As might be expected, the results show a considerable variation. In Fig. 16, for instance, there is an occasional agreement in some of the brief waves recorded at two pairs of electrodes 3 mm. from one another, but in a good deal of the record there is no agreement at all. This shows that there is no likelihood of an electrical spread from one pair of electrodes to the other and makes the occasional agreement more significant.

Fig. 16. Occasional agreement of brief waves at electrodes 3 mm. apart. Rabbit, C. and E. Pointed gelatin electrodes arranged in pairs, I, II and III (see diagram) with 1 mm. separation between the electrodes of each pair. I is 3 mm. from II and II is 4 mm. from III. In the first outburst the waves at I and II show some agreement, but there is very little in the later part of the record. No agreement at III. Time marker gives 1/20 sec.

From this and many similar records it appears that in the normal activity of the anæsthetized brain a synchronous pulsation does not cover an area of more than 3–4 mm. diam. and is often much more restricted. An area of 3 mm. diam. might be covered by the dendrites of a single nerve cell, but there is no reason to doubt that each brief wave is the product of a group rather than a unit. Synchronized action occurs, apart from gross injury, in different regions of the nervous system, *e.g.* in the motor centres of the cord, in the retina, the optic ganglion of *Dytiscus*, etc. It is most evident when there is an intense, uniform excitation of the whole region, and it might well occur in smaller areas if these were uniformly excited.

(*b*) *The slow waves. Rise and fall of activity in larger areas.* If the slow waves are due to summated brief waves from many neurones it follows that there must be a general agreement in the periods of rest and activity over considerable areas, although the neurones in them are not pulsating

in phase. The mere existence of a potential gradient shows, of course, that the activity is not uniform throughout the cortex. Indeed the size of the waves will be a measure of the gradient of activity along the line joining the electrodes, and by recording simultaneously at several points we can gain some idea of the distribution of activity from moment to moment.

It is found that the distribution is complex in light and moderate anæsthesia but simpler in deep anæsthesia; with light anæsthesia the waves at three pairs of electrodes 5 mm. or more apart show little or no

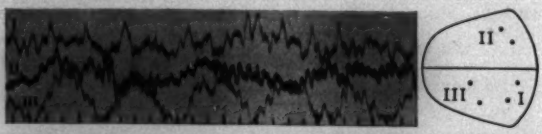

Fig. 17. Lack of agreement in activity at different points in moderate C. and E. anæsthesia. Thread electrodes, 4 mm. separation. Arranged as in diagram with 10 mm. between I and II and 5 mm. between II and III. Note continuous series of brief waves, and slow waves with regular 4 per sec. rhythm at electrodes III only. Time marker gives 1/20 sec.

Fig. 18. Agreement of active periods in deep urethane anæsthesia (rabbit). Electrodes 3 mm separation arranged as in diagram. 15 mm. between I and II, 5 mm. between II and III. Time marker gives 1/10 sec.

correspondence (Fig. 17), but in deep anæsthesia they agree very closely. In the typical response under urethane the slow waves appear almost simultaneously at points 1 cm. or more apart (Fig. 18), and in deep C. and E. anæsthesia there is a regular cycle of rest and activity which is equally widespread (Fig. 19). This cycle has a period of 1–2 sec. and appears only when the anæsthesia is strong enough to threaten respiratory failure if its administration is prolonged. The regular waves at 3–4 per sec. which occur in lighter C. and E. anæsthesia are usually confined to one pair of electrodes, or appear with an independent rhythm at different points (Fig. 17).

Regular beating of this kind could scarcely occur except in a cortex undisturbed by external influences, and it is found that if sensory stimulation produces any effect at all that effect is to break up the rhythm. This is best seen in urethane anæsthesia light enough to allow a slight reflex contraction of the leg muscles when the foot is pinched. Illustrations of this are given in Fig. 20, and it will be seen that pinching the foot abolishes the slow rhythms and increases the prominence of the brief waves, which

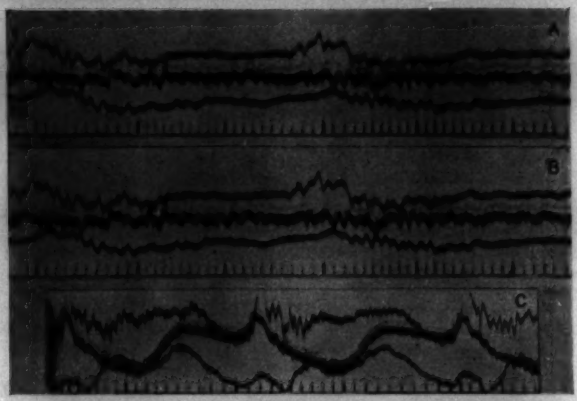

Fig. 19. Agreement of active periods in deep C. and E. anæsthesia (rabbit). A and B, pointed gelatin electrodes 1 mm. apart arranged in pairs as in Fig. 16 with 3 mm. between I and II and 4 mm. between II and III. Outbursts of brief waves at 1½ sec. intervals. C, another animal. Thread electrodes 4 mm. apart arranged in pairs as in Fig. 17. Outbursts at 1 sec. intervals. Note the presence of slow waves in C (electrodes 4 mm. apart) and their absence in A and B (electrodes 1 mm. apart). Time marker gives 1/20 sec.

now appear in a continuous outburst. In some records the slow waves have returned at a higher frequency towards the end of the stimulation.

It will be clear from Figs. 17–19 that in deep anæsthesia periodic waves of activity may sweep over the whole cortex (or rather over its dorsal surface), and that in lighter anæsthesia one or more regions may beat rhythmically at 3–4 per sec. Waves of the same kind occur in the cat as well as in the rabbit, though not with such uniformity. They are not in phase with respiration or the heart beat and are not appreciably altered by changing the blood-pressure or the composition of the air breathed by the animal. They may depend on subcortical mechanisms, but all that

can be said at the moment is that they show that the respiratory centre is not the only region of the brain which has a tendency to slow, rhythmic beating. The rhythmic movements of narcosis progression described by Graham Brown [1914] are probably due to beats of the same kind. Indeed, Graham Brown's suggestion of inherent rhythms modified by afferent control seems to be well illustrated by records such as those in Fig. 20.

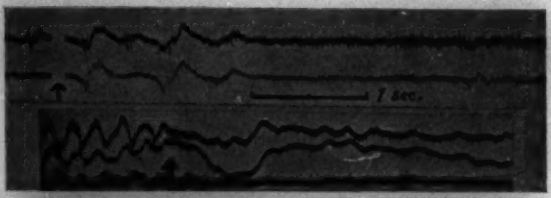

Fig. 20. Rhythmic outbursts cease on sensory stimulation in light anæsthesia. Both records are made with earthed amplifiers, not with the balanced type. The agreement in the waves may be due to the common earth leads. A, rabbit, urethane. Pinching foot at arrow. The slow waves give place to a continuous series of rapid oscillations. B, another animal. Pinching foot at arrow.

Transitional waves. (*a*) *Due to injury.* In the spontaneous activity of the anæsthetized brain there is no evidence of synchronous pulsation spreading over areas of more than 4 mm. diam. In the injury discharges much larger areas may be involved and there is often a progressive increase as the discharge proceeds. Thus the ease with which the pulsation of one neurone spreads to another can vary with the condition of the cortex. In certain conditions the spread takes place with abnormal ease, and a single pulsation may travel like a ripple over the surface of the brain, giving rise to a characteristic potential change.

This type of activity is often seen as a sequel to an intense injury discharge and it is invariably found with certain convulsant drugs. It leads to the production of very large potential waves, often with a smooth contour, representing a transition between the slow and the brief type.

The simplest case to take is that produced by injury. As a rule the rapid pulsation ceases by becoming irregular after the frequency has fallen below 35 per sec.; in some records, however, very large waves appear from time to time during the discharge and ultimately these become its most prominent feature. They may occur singly at intervals of

$\frac{1}{2}$ sec. or so, or in groups of three or four. Their potentials may be as great as 5 millivolts and they appear over a wide area. Examples of them are given in Fig. 21. The waves usually begin as simple monophasic spikes differing only in size from the other waves in the series (Fig. 21 C and D), but ultimately they may become di- or triphasic, or may present multiple crests and troughs as in Fig. 21 B. This change in appearance seems to be

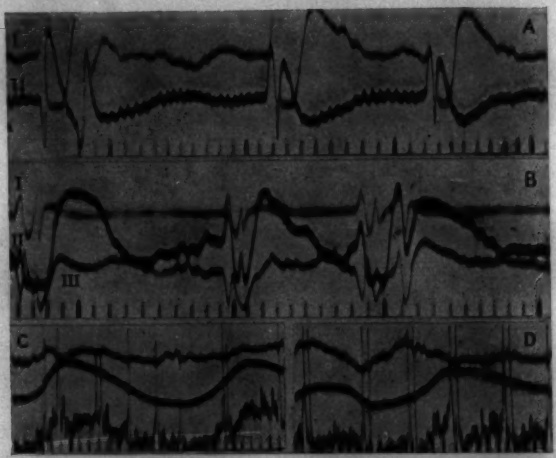

Fig. 21. "Transitional" waves due to injury. A, rabbit, urethane. Earthed amplifiers. Later stage of injury discharge with three large waves and small oscillations at 40 per sec. B, rabbit, urethane. Balanced amplifiers. Electrodes arranged as in Fig. 12. I 1 mm. apart, II 9 mm. and III 16 mm. Sequel to prolonged injury discharge. Note absence of slow waves at I. C and D, rabbit, urethane. Earthed amplifiers. Lowest record shows development of large transitional waves (monophasic) occurring singly at first and then in couples. Time marker gives 1/20 sec.

due to the waves or groups of waves spreading at a finite rate through the cortex so that the region of maximum negativity lies now under one electrode and now under the other. But the conduction takes place without the waves losing their individuality and an almost identical pattern of activity may appear at points 10 mm. or more apart.

The development of these large waves out of the brief pulsations of the injury discharge shows how a broader contour and a diphasic form may result from a pulsation which spreads over the cortex instead of being

confined to a small area. In the discharges produced by convulsant drugs we find the same kind of conducted pulsation and can sometimes follow the change from the transitional type into the usual complex of slow and brief waves.

(*b*) *Convulsant drugs, thujone.* Strychnine produces widespread synchronous activity in the nervous system and might therefore be expected to give a cortical discharge made up of widespread pulsations. Fischer [1933 *a*], Bartley [1933] and others have recorded the cortical response, and their records show a succession of waves of very large potential repeated as rapidly as 25 per sec., or occurring singly or in groups at lower frequencies. At the higher frequencies they have the simple contour of the waves in an injury discharge and at lower frequencies they resemble the large "transitional" injury waves shown above. Fischer has used other convulsant drugs, picrotoxin, caffeine, etc., with the same results. In our own experiments with convulsants we have generally used thujone, the active principle of wormwood oil. In the unanæsthetized animal thujone produces convulsions which are said to resemble those of epilepsy. In an animal deeply anæsthetized with urethane there is usually no motor effect, but there is an obvious counterpart of the convulsion in the potential record from the cortex.

We have used an emulsion of thujone in olive oil and gum arabic solution injected into the femoral vein. A dose of 0·5 c.c. of a 1 in 10 emulsion is enough to produce the typical cortical discharge in a rabbit under urethane. As a rule the first sign of the effect is that the slow urethane waves disappear: then after an interval of apparent inactivity there is a more rapid series of slow waves, each with a small group of brief waves superimposed. Very soon, instead of a group of brief waves on a slow deflection, we find a sudden large oscillation, wider and more complex in form than that of the usual brief waves, but no longer clearly divisible into fast and slow components. A series of records showing the evolution of a thujone discharge is given in Fig. 22. To avoid confusion only one oscillograph tracing is shown in the figure, although three simultaneous tracings were made from different points. It will be seen that the discharge works up into a series of large diphasic waves of simple contour; the wave form changes from time to time and ultimately the discharge stops suddenly and completely.

In this particular record the frequency of the large waves is never greater than 10 per sec., but it may reach 20 per sec. or more with larger doses, and the large beats are sometimes preceded or followed by smaller 50 per sec. oscillations.

The factors underlying the wave form and the changes it undergoes can be studied by simultaneous recording at different points. At the height of the discharge the whole surface of the cortex pulsates with the same rhythm though occasionally a beat is missed at one pair of elec-

Fig. 22. Evolution of discharge due to thujone in a rabbit under urethane. Portions of a continuous record, intervals of 2–3 sec. between successive strips. Pointed gelatin electrodes 1 mm. apart on surface of cortex. Three simultaneous records were made from different points but the tracings of oscillographs I and III have been painted over to show II more clearly. The largest deflections represent potential changes of about 5 mv.

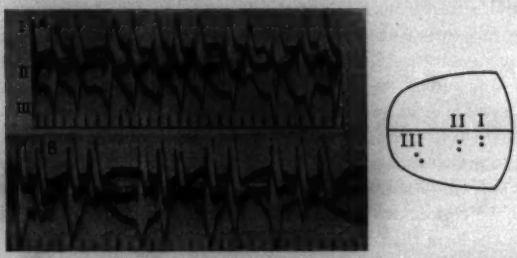

Fig. 23. Record of thujone discharge from three points on the cortex, showing agreement of waves. A, near the beginning of the discharge, waves 10 per sec. One wave is missed at III (8 mm. from II), but otherwise there is complete agreement. B, later; I and II still agree, but more waves are missed at III. Gelatin electrodes 1 mm. apart, 4 mm. between I and II, 8 mm. between II and III. Time marker gives 1/20 sec.

trodes (Fig. 23). More information can be gained, not from three pairs of electrodes a long distance from one another, but from four electrodes in line (*W*, *X*, *Y*, *Z*, Fig. 24) with the three recording systems between adjacent electrodes. With such an arrangement we can map out the potential distribution along the line and see how it changes from moment

to moment. Two characteristic records from the same discharge are given in Fig. 24. In Fig. 24 A the waves have a broad contour, they are diphasic and the maximum negativity of *W* relative to *X* occurs about 0·025 sec.

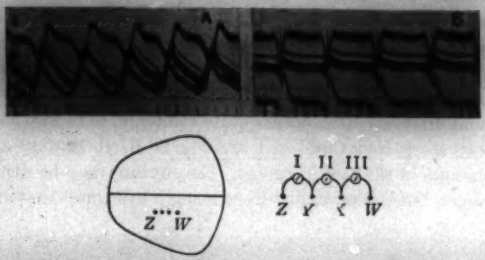

Fig. 24. Thujone waves recorded at four electrodes in line, 1 mm. apart. Two strips from a continuous record. Rabbit, urethane (deep). 0·5 c.c. of 1 in 10 thujone emulsion into femoral vein. Electrodes etc. arranged as in diagram, at *W*, *X*, *Y*, *Z*. Negativity of *W* relative to *X*, *X* to *Y* and *Y* to *Z* gives a downward deflection. In A the wave is simple and travels slowly from *W* to *Z*, in B it is complex and travels much more rapidly, see analysis in Fig. 27. Time marker gives 1/20 sec.

Fig. 25. Thujone waves with four electrodes in line 1 mm. apart, showing progressive changes in rate and direction. Electrodes arranged as in Fig. 24. In A the wave starts from *Z*, and successive waves spread more and more slowly until the discharge ceases. In B the wave starts from *Z* at the beginning of the record and has come to start from *W* by the end of it. Time marker gives 1/20 sec.

before that between *X* and *Y*, and 0·05 sec. before the maximum between *Y* and *Z*. In Fig. 24 B the waves are sharper and more complex and they occur almost together in each recording system. It follows that in the upper record a single pulse of activity begins at *W* and spreads relatively slowly to *Z*, whereas in the lower there are several pulses and the rate of

spread is very much more rapid. In Fig. 24 the wave form remains very nearly constant throughout the record, but there is often a gradual change from moment to moment. Thus in Fig. 25 A as the frequency of the beat declines the activity spreads more and more slowly along the line (starting in this case from the *Z* end). In Fig. 25 B there is a complete reversal during the seven waves in the record: the activity starts from the *Z* end in the first beat, in the middle of the series it appears first in the region *XY* and by the end it has come to spread down the line from *W* to *Z*. The change in velocity and direction often occurs quite suddenly, as in Fig. 26 A, where a single beat starts from the *W* end, and Fig. 26 B, where there are several abrupt changes in the rate of spread. The rhythm may be disturbed by these changes, but it is never disturbed enough to suggest that an entirely

Fig. 26. Sudden changes in direction and rate of spread. Electrodes arranged as in Fig. 24. In A a single beat starts from *Z*. In B the rate of spread changes abruptly from time to time. Time marker gives 1/20 sec.

independent source of activity has taken charge. The potential gradients between the four electrodes at different moments are represented, for a few typical beats, in Fig. 27. They show that the beat sometimes involves a double pulsation in part of its course and that it travels at a variable rate, sometimes in one direction and sometimes in another. The change in rate may well be due simply to the change in the direction of the wave, for if it travels in a direction parallel to the line joining the electrodes the rate of conduction will appear much slower than if the direction is nearly at right angles. The rate in Fig. 24 A is of the order of 10 cm. per sec., but the uncertainty as to the direction of travel makes it impossible to say how far this applies to all the beats.

The sudden or gradual shifts in direction are not surprising if we assume that the cortex behaves under thujone like a mass of cells as closely linked as are the muscle fibres of the heart. Spontaneous beats

may arise now from one point and now from another, or the origin may shift progressively without affecting the rhythm. Similar changes often take place in the spontaneous beats produced in skeletal muscle fibre bathed in NaCl solution. A record illustrating this is given in Fig. 28, and it will be seen that the origin of the beat shifts gradually during the discharge so that the action potentials come to be reversed in phase, as in the

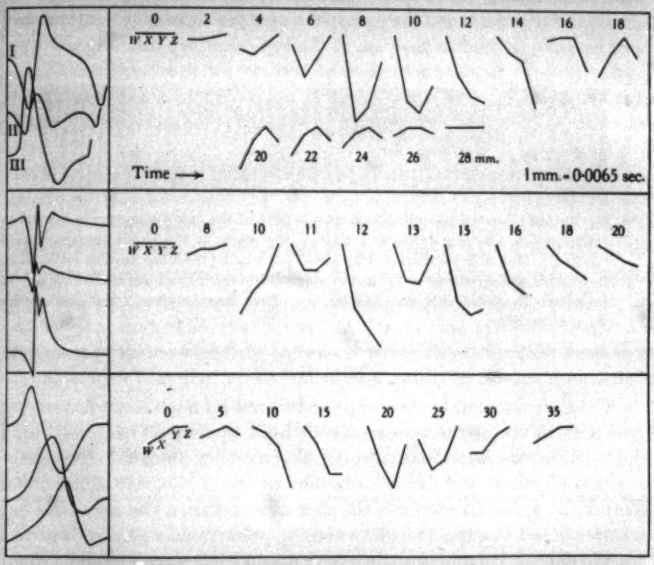

Fig. 27. Relative potentials at the four electrodes, *W*, *X*, *Y* and *Z*, at different periods during the passage of a wave. Tracings of the oscillograph record are given on the left. The potential distribution is given at various intervals after the beginning of the wave.

cortical record in Fig. 25 B. It is possible that the cortical beats arise as the result of impulses coming from subcortical structures, though there is evidence to show that in certain forms of experimental epilepsy the fit spreads by conduction in the cortex. There is no reason to suppose, however, that the thujone waves will always spread uniformly. Some areas may be more fatigued than others, and these may form occasional islands of inactivity round which one wave must travel though the next may find them responsive again.

As the effect of the drug wears off there is a gradual return to the usual complex of brief and slow waves typical of urethane—as though the syncytium were breaking up again into smaller and smaller groups.

For the present argument the chief interest of the thujone discharge is that it gives no evidence of distinct brief and slow components in the cortical response. The diphasic form of the waves is obviously due, as in a nerve or muscle fibre, to the passage of the active region from one electrode to the next, and the monophasic potentials in each neurone may well be quite as brief as they are in the typical injury discharge.

Fig. 28. Record of action potentials in a muscle fibre of the frog's sartorius immersed in NaCl solution, showing progressive shift in the origin of the waves (compare with Fig. 25 B). Muscle in bath divided by a slot, leads taken from the fluid on either side. There is a gradual reversal of the waves as the origin shifts from one side of the slot to the other. Maximum frequency 44 per sec. Time marker gives 1/4 sec. Battery-coupled amplifier.

Discussion.

The main outcome of these experiments has been to satisfy us that the potential waves in the cortex are all built up out of relatively brief pulsations in the individual neurones, that the slow potential changes are summation effects and have no counterpart in the response of the units. We set out with a bias towards the opposite conclusion and for a time our results seemed to support it. The turning point came when we began to use the balanced amplifier system for independent recording from several points. This gave conclusive evidence that the slow waves needed large areas for their development and showed how the earlier results were vitiated by the use of diffuse earthed leads.

Although the time relations found in the injury discharge have been surprisingly constant in all our experiments we can scarcely conclude that the response of all the cortical neurones is a series of brief waves of identical duration. The constant form of the action potential in a nerve trunk seemed to indicate uniformity in the constituent fibres until Gasser and Erlanger [1927] showed how widely the units might differ. There may be as great or greater differences in the units of the cortex, though, as in nerve, the major part of the electric effect is probably due to a more or

less homogeneous group. Again, we cannot be sure that the pulsations of a given cortical unit do not vary from moment to moment, *e.g.* with fatigue. All that can be said is that the response is probably a simple monophasic spike which occupies not much more than 0·1 sec. and not much less than 0·01 sec. We cannot say that the responses do not vary in size, but all the variations we have found could be explained as due to changes in the number of units in action, and it can often be shown that an exceptionally large wave is in fact the product of a greater number of neurones.

Thus the cortical waves seem to be built up out of relatively simple components, rhythmic pulsations in the nerve cells varying in frequency like the impulses in a nerve fibre but not necessarily varying in any other way. The complexity of the cortical response is due to the complexity of the structure which gives rise to it, not to a more variable type of activity in the units. This means that we must abandon the hope that the slow potential changes in the cortex might be an index of slow change of polarization in the nerve cells and so an index of the rise and fall of the excitatory state which preludes activity. Slow changes may occur, indeed the work of Gasser and Graham [1933] on the after-potentials in the spinal cord and of Eccles [1934] on the cervical sympathetic ganglia makes it probable that they do occur—but in the cortex they must be so much smaller than the "spike" potentials that in the normal spontaneous activity they cannot be detected.

This leads at once to the further question as to the origin of slow potential changes in other parts of the nervous system. The slow respiratory waves in the brain stem of the goldfish [Adrian and Buytendijk, 1931], in the thoracic and optic ganglia of *Dytiscus* [Adrian, 1932] and in the retina have all been considered as evidence favouring the idea of slow potential changes in individual nerve cells. Their smooth contour was the main reason for supposing that they were not summation effects, but the smooth contour of many of the slow waves in the cortex makes it very doubtful if the argument can be sustained. The fact that the potential wave of the nerve cell is much less abrupt than that of the axon is probably a sufficient explanation even for the slow changes in insect ganglia.

Effects of anæsthesia.

It must be admitted that all our observations on the cortex in animals have been made under general anæsthesia. There was no lack of electrical activity, but in the unanæsthetized brain it might turn out to be an

activity of an entirely different order. As regards the individual neurones, however, there is no evidence to suggest any radical difference. Fischer, Kornmüller and Tönnies have worked on animals immobilized by curare, and their records have shown the same mixture of slow and brief waves, and Bartley and Bishop [1933] state that the only definite change as anæsthesia develops is a slight reduction in the magnitude of the potential waves. With light as opposed to deep anæsthesia we have found a more continuous and more irregular succession of waves with a more complete lack of correspondence in the waves at different points. In this condition the cortex may be accessible in a slight degree to afferent impulses, for stimulation may change the nature of the response for a time (*vide* Fig. 20). It is probable, then, that in the lightly anæsthetized and still more in the unanæsthetized brain afferent impulses will always break up any tendency to uniform activity. With deeper anæsthesia there is less to interfere with the spontaneous activity of the nerve cells, and we find more regular waves and greater uniformity in different regions.

With C. and E. there is a gradual diminution in the size of the waves as the anæsthesia deepens, but the final disappearance of activity occurs abruptly within a few seconds of the failure of respiration (either before or after) and coincides with the blanching of the cortex. It seems to be due not so much to the direct action of the anæsthetic on the cortex as to the sudden fall of blood-pressure. As soon as the circulation is restored (by artificial respiration and massage of the chest) the potential waves reappear, often as large rhythmical outbursts with intervals of complete rest, returning later to the more usual type of response. Failure of the oxygen supply is thus the most important factor in the failure of the electrical activity. The dependence on oxygen supply can also be shown by giving a few breaths of nitrogen or by compressing the vertebral arteries (the carotids having been ligatured previously).

It is remarkable to find so much electrical activity in the anæsthetized cortex and still more to find that it is not so much a random activity of independent units as a series of disturbances spreading over large areas. With thujone, for instance, the whole cortex may pulsate with the same rhythm, and waves seem to be conducted freely in all directions: this may occur in an animal in urethane anæsthesia deep enough to prevent any movement during the thujone discharge and to prevent any sensory stimulation from reaching the cortex and modifying its response. All this supports the suggestion made by Bartley and Bishop [1933] that the chief effect of the anæsthetic is on the afferent and efferent pathways

rather than on the cortex itself, but more evidence is needed before any certain conclusion can be reached. It is at least clear that the same physical laws will govern the potential distribution in the unanæsthetized brain, so that there will be the same opportunity for summation effects from large areas.

To sum up—our evidence points to the conclusion that the electrical activity of the cortex is due to events of the same character as those taking place in active nerve or muscle fibre, *i.e.* to successions of brief action potentials repeated rhythmically with a frequency which can vary within wide limits. Arguing from nerve and muscle fibre we may expect that there is little or no gradation in the electrical response of each unit apart from the change in frequency. The evidence on this point is scanty, and all that can be said is that an increase in the size of the brief waves is usually found to involve an increase in the area contributing to the wave. But this, in itself, is an important distinction between the behaviour of the cortical units and those of a nerve trunk. In the latter the units, if undamaged, are completely independent. In the cortex they pulsate in groups small or large. With intense excitation rapid synchronous pulsations may extend over an area of 5 mm. or more diameter, and from time to time a single beat may travel through the entire cortex. In fact with a focus of intense excitation (*e.g.* from injury) or under the action of a convulsant drug the mass of cortical neurones behaves more like the linked fibres of the heart muscle than the independent fibres of a skeletal muscle or a nerve. In the unanæsthetized brain the constant arrival of afferent impulses at different points must break up the tendency to synchronous action. It must be remembered, however, that we are dealing throughout with brains which are very small compared to that of man, and that the largest areas of synchronous action which we have found would occupy only a very small fraction of the human cortex.

Summary.

1. Potential changes recorded from the cortex of the anæsthetized rabbit are roughly divisible into large slow waves and smaller brief waves superimposed on the large. We have tried to find out what kind of electrical activity takes place in the individual neurones of the cortex to give rise to these waves.

2. In the light anæsthesia the waves occur irregularly, but in deeper anæsthesia there is much greater regularity, the slow waves occurring at intervals of 1–2 sec. in deep C. and E. or urethane and at 3–4 per sec. in

moderate C. and E. anæsthesia. In lighter anæsthesia sensory stimulation often abolishes the rhythmic beat and substitutes a continuous train of brief waves.

3. Injury to the cortex produces a characteristic discharge of brief waves in a rapid sinusoidal oscillation. The frequency falls gradually from a maximum of 100–60 per sec. to a minimum of 40–35 per sec. Below this the discharge becomes irregular. By recording simultaneously with three pairs of electrodes it can be shown that the potential changes in the injury discharge often occur over an area 5 mm. or more in diameter. They are probably due to synchronous pulsation in a large number of cortical units.

4. The injury discharge shows that the cortical neurones may develop a pulsating activity, like that of a nerve or muscle fibre. The brief waves in the response of the uninjured cortex are due to an activity of the same kind. The problem is to decide whether the slow waves represent a different kind of activity, or whether they are merely due to a summation of the brief waves.

5. If the slow waves are summation effects they should be present when the electrodes are widely separated, but absent or very small when they are close together. It is found that they are only present when the electrodes are arranged so that large areas can contribute to the potential changes. With restricted electrodes only the brief waves appear. It is concluded that the response of the individual neurone is a brief pulsation and not a slow change.

6. In the anæsthetized, but uninjured, brain the neurones tend to pulsate in small groups covering an area not more than 3–4 mm. diam. Periodic waves of activity may spread over the whole cortex in deep anæsthesia, but the neurones which take part in it still react in small groups which pulsate out of phase with one another.

7. After severe injury or under the action of a convulsant drug (thujone) a pulsation may spread widely through the cortex. The potential waves are then of "transitional" type and are no longer divisible into slow and brief components. An analysis of the waves in the thujone discharge shows that the pulsations often change their direction of travel. The cortex behaves as a freely conducting mass with beats starting now from one focus and now from another. Each beat probably represents a single brief pulsation, or rapid series of pulsations, in the individual neurones.

8. The effects of anæsthetics are discussed. It is suggested that their main effect is on the afferent and efferent pathways rather than on the

cortical neurones themselves, and that these are not likely to show any different type of electrical activity in the unanæsthetized brain.

9. It is concluded that the activity of the cortical neurones consists of a series of brief pulsations which can vary in frequency but probably not in size. Their time relations are not as rapid as those of the action potential in a nerve fibre. Except with intense excitation the frequency of the discharge does not rise much higher than 50 per sec.

It has not been possible to detect very slow changes of potential apart from summation effects. Gradual changes of polarization may occur, but the electrical effects must be much smaller than those due to the brief action potentials. Many of the slow potential changes in other preparations of the central nervous system may be due, like the slow waves in the cortex, to a summation of the brief responses of individual neurones.

The expenses of this work were defrayed by a grant from the Foulerton Committee of the Royal Society.

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612.63.031.1:612.492

THE FORMATION OF THE CORPUS LUTEUM IS DEPENDENT ON THE ANTERIOR PITUITARY LOBE, AND NOT ON THE MATURING OVUM. THE FERTILIZED OVUM AND HORMONES[1].

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(*Received March* 19, 1934.)

In earlier experiments [1926] I have shown that the impulse for ovarian function does not lie in the ovary itself but emanates from the anterior lobe of the pituitary body. As a result of the implantation of the tiniest pieces of anterior lobe tissue, either human or animal, male or female, it was possible to initiate the ovarian function of an infantile animal and to restore that of a senile animal. The reactions (H.V.R. I–III synonym with A.P.R. I–III) produced in the ovary were employed as a test for the presence of gonadotropic anterior pituitary lobe hormone [Zondek and Aschheim, 1927]. With this test I was able to prepare the gonadotropic hormone (prolan). By means of prolan we can influence the ripening of the follicle as well as the formation of the corpus luteum. The problem as to whether the development of follicular growth and the production of folliculin on the one hand and the formation of the corpus luteum and the production of progestin on the other hand are induced by two different kinds of prolan (A and B) or whether it is only a question of the difference in the quantity of prolan secreted will not be discussed in this paper.

From the effects of the gonadotropic anterior pituitary lobe hormone it has been inferred that the anterior pituitary lobe is the motor of sexual function, and that the anterior lobe is the place where the general and non-specific sex hormones are produced. The earlier view that the ovum regulates the hormonic processes of the ovary, *i.e.* that the impulse for the Lovarian function emanates from the ovum [Robert Meyer, 1913], could not be easily reconciled with the findings concerning the gonadotropic

[1] I am indebted to Dr Bernard Homa, London, for the translation.

influence of the anterior pituitary lobe hormone. As a result of experimental investigations [Zondek, 1931] I have concluded as follows: "Ovum and hormone are coordinated in function, are not interdependent, but are both governed by the anterior pituitary lobe." That the gonadotropic anterior pituitary lobe hormone produces ripening of the follicle is now universally recognized. On the other hand, the question is again being discussed as to whether luteinization is caused by the anterior lobe hormone or whether a hormone is produced in the maturing ovum which stimulates the formation of the corpus luteum. This latter hypothesis [Clauberg, 1933; Schoeller, 1934] is not proved experimentally by its authors. According to this theory a very important hormonic function would devolve upon the ripening ovum, viz. the development of the corpus luteum following rupture of the follicle and the production of progestin. The following experiments show that the ovum is of no objective importance to the formation of the corpus luteum.

I. Experiments on the ovary of the bat during hibernation.

During hibernation the ovary is in a state of functional rest. By means of serial sections it was found that whereas one ovary might contain only small follicles the other might have one or at most two enlarged follicles. These follicles rupture in the spring and the ova are immediately fertilized, as copulation has already taken place before hibernation. The sperm remains in the uterus throughout the winter. The ovaries of the hibernating bat are, as I have recently reported [Zondek, 1933], especially suitable for the study of the action of prolan. It is possible to cause follicular rupture and thereby pregnancy during the winter by means of prolan. If large doses of prolan are injected several corpora lutea can be produced (4–6 in one ovary), an effect which never occurs physiologically, as the bat produces one or at most two offspring. These corpora lutea are well vascularized and are functionally active as may be seen by the effect upon the mucous membrane of the uterus. As a result of large doses of prolan there is no rupture of the follicle, only the formation of corpora lutea atretica, in which the ovum may be pressed to one side. There is no sign of maturation to be seen in the ovum. During the rapid development of the corpora lutea as a result of large prolan doses, the ovum of the bat may be destroyed; in spite of this a functioning corpus luteum is formed. The ovum is therefore not essential to the development of the corpus luteum. Against these experiments it might be argued that although the ovum gets destroyed it has already produced a hormone which stimulates the formation of the corpus luteum. It is therefore necessary to prove by

direct means that the development of the corpus luteum occurs also after the ovum has been removed from the follicle. These experiments may be carried out on rabbits.

II. Experiments on the removal of the ovum from the follicle of the rabbit.

We find in the ovary of a sexually mature rabbit several large follicles ready to rupture. As Heape [1905] has shown, follicular rupture in the rabbit is brought about by copulation. The experiments of Fee and Parkes [1929] have shown that the stimulus for follicular rupture occurs *via* the anterior pituitary lobe. If hypophysectomy of the rabbit is performed within 1 hour of coitus, ovulation will be prevented; if, however, hypophysectomy is carried out after 1 hour following copulation, ovulation commences from 10 to 12 hours later and corpora lutea result. From these experiments it follows that a substance in the hypophysis is brought into action which causes rupture of the follicle and the formation of a corpus luteum. If the hypophysectomy is performed after 1 hour following copulation, prolan is already circulating in the organism and in consequence follicular rupture and corpus luteum formation take place.

Procedure. The proof that the ovum does not influence the formation of the corpus luteum can be adduced in a direct manner by removing the ova from the ripened follicles of a sexually mature rabbit. For this purpose I punctured the follicle with a fine cannula and aspirated the liquor folliculi together with the ovum. The material withdrawn from the follicles was put into formalin and centrifugalized. The ova were to be found in the sediment, this was embedded in paraffin wax, and the ova could be easily demonstrated in microscopic section (Fig. 1). If an ovary so treated is removed 24 hours after and examined some blood may be seen in the follicular cavity as a result of the puncture; ova are no longer present. In this way follicles may be obtained that are deprived of ova (Fig. 2). In some cases I injected alcohol into the follicular cavities after withdrawing the ova, with the object of destroying any remaining particles of ovum. The treatment with alcohol is, however, superfluous as the aspiration withdraws the ovum from the follicular cavity *in toto*, surrounded by the membrana granulosa. The injection of alcohol does not, however, prevent the subsequent development of the corpus luteum.

In order to make the follicle recognizable I cut away the cupola of the follicle in several cases, after aspirating the ovum, with a fine pair of scissors, and thus prepared an open follicle deprived of its ovum.

Since one could argue that such a follicle might be stimulated to

produce a corpus luteum by a maturing ovum contained in another follicle, I carried out the experiment with only one follicle and that containing no ovum. For this purpose one ovary was extirpated. In the other ovary a mature follicle situated in the middle of the gland was punctured, the ovum aspirated and then the ovarian tissue on both surfaces was resected so that only the one punctured follicle remained. It is necessary to take care when resecting the ovarian tissue that the follicle left behind is not deprived of its blood supply. This is easily carried out in practice.

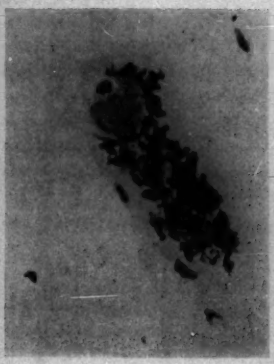

Fig. 1.

Fig. 2.

Fig. 1. Isolated ovum, withdrawn from follicle.

Fig. 2. Ovumless follicle. Follicle punctured, hæmorrhages in the follicular cavity following aspiration of the ovum.

Results. The rabbits were given 24 hours after the operation intravenous injections of prolan from pregnancy urine. I injected a large dose of prolan, viz. 1 c.c. = 1000 R.E. The animals were examined 24–48 hours after the injection. In each case the result was the same: the luteinization of the follicle occurred exactly as that of a follicle containing an ovum. I found all the stages from the commencement of luteinization (Fig. 3) to the completely formed corpus luteum (Fig. 4). The follicles with cupolas cut away gave particularly interesting pictures. The follicle is open, and one observes some blood resulting from the puncture, and nearby the

Fig. 3. Partial luteinization of the ovumless follicle after injection of prolan. The cupola of the follicle is open, the cavity still contains blood following aspiration of the ovum.

Fig. 4. Corpus luteum developed from an ovumless follicle after injection of prolan. Cupola open.

transformation of the follicular cells into lutein cells (Fig. 3). If only one follicle with no ovum in it is left behind this, too, is converted into a corpus luteum.

The experiments therefore show that the ovum is of no importance for the formation of the corpus luteum. By the introduction of gonadotropic hormone we are able to produce the development of the corpus luteum whether the maturing ovum is present in the follicle or not. The impulse for the formation of the corpus luteum cannot therefore emanate from the maturing ovum. The theories which ascribe to the ovum the development of a hormonic substance for the production of the corpus luteum may thus be contradicted. The formation of the corpus luteum is therefore, as with the ripening of the follicle and ovum, dependent upon the gonadotropic hormone of the anterior pituitary lobe (prolan).

I have made a short report of these experiments in the Colloquium of the Biochemical Institute of Stockholm (February 5, 1934). On that occasion there was a report by Westman concerning experiments which were carried out quite independently of me and which also dealt with the development of the corpus luteum in a follicle containing no ovum. The method employed by Westman was somewhat different from my own. Our experiments, however, gave the same result, they both showed that the formation of the corpus luteum is quite independent of the ovum.

The fertilized ovum and hormones.

The present experiments as well as earlier ones show that the maturing ovum is of no significance either for the ripening of the follicle and the production of folliculin (first generative phase) or for the development of the corpus luteum and the production of progestin (second generative phase). On the other hand the ovum is of definite importance in hormonic processes once it is fertilized. Both in human beings and in all other mammals there is the regular occurrence of a persistent corpus luteum which consequently prevents any further ripening of ova. As Corner and Allen [1929] have shown, the secretion of progestin from the corpus luteum graviditatis is essential to the life of the young fertilized ovum.

During the embedding of the ovum, at the time when contact with the material circulation is established, there is a great over-production of folliculin and prolan [Aschheim and Zondek, 1927–28]. This occurs only in man and apes, but not in other mammals. The greatly increased production and excretion of folliculin and prolan is neither preliminary conditional to nor consequent upon every fertilization. Especially interesting are the conditions during pregnancy in the equines (horse, ass,

zebra). In these I found [Zondek, 1930] a particularly increased production of folliculin, 100,000 mouse units of folliculin are excreted per litre of urine. This increased production of folliculin occurs in the equines as in the primates during the whole of pregnancy. On the other hand, the increased production of prolan, as Cole and Hart [1930] have shown, is limited to a certain period of pregnancy (43–100 days). The horse during pregnancy—contrary to the primates—is therefore only temporarily polyhormonic as far as prolan is concerned. I mention these differences in order to show that the great changes in hormonic content which set in after the embedding of the fertilized ovum are not the same in all mammals. We can only determine the changes as facts, but we must be very careful in their interpretation as after events of fertilization. All the more so since the mass production and mass excretion of œstrogenic hormone which has hitherto been determined only in cases of pregnancy can be observed apart from such cases and curiously enough even in a male animal. As I have recently reported [Zondek, 1934], the largest quantities of œstrogenic hormone are excreted in the urine of the stallion (170,000 m.u. per litre) and in that of other male equines (zebra 40,000, ass 4000 m.u. per litre). In castrated horses (geldings) and in young sexually immature horses (colts) only very small quantities of œstrogenic hormone are found (0·2 p.c.). It has been demonstrated that the œstrogenic hormone is produced in the testis of the stallion. The testis of the horse has shown itself to be the richest tissue containing œstrogenic hormone so far known. Both testes of a stallion contain 4–5 times as much hormone as a human or horse's placenta.

The significance of the fertilized ovum for hormonic processes lies in the fact that after fertilization the corpus luteum continues to exist and continues to produce progestin. Since the formation of the corpus luteum, as now shown, is dependent only upon the gonadotropic hormone of the anterior pituitary lobe, it must be accepted that the fertilized ovum exerts an impulse on the anterior lobe of the hypophysis which by means of the alteration of its production of prolan controls the continued existence of the corpus luteum. In this sense I agree with the opinion of Robert Meyer concerning the significance of the ovum for the functional process.

Summary.

1. Experiments on the ovary of the hibernating bat show that corpora lutea may be produced by means of prolan, although the ovum is pressed to one side or may be destroyed. From this it is already apparent that the formation of the corpus luteum cannot be dependent upon the ovum.

2. Direct proof may be given from the follicles of the sexually mature rabbit from which the ova are withdrawn by aspiration. After the injection of prolan such a follicle is converted into a corpus luteum. For the production of a corpus luteum it makes no difference whether an ovum is present in the follicle or not.

3. The formation of the corpus luteum can therefore not be dependent upon the ovum. The gonadotropic hormones of the anterior pituitary lobe effect ripening of the follicle and ovum, follicular rupture and development of the corpus luteum. The ovum plays no part in these hormonic events.

4. On the other hand, after it is fertilized, the ovum assumes a definite significance for the hormonic process. As a rule there follows the persistence of the corpus luteum which must be controlled by a reaction of the fertilized ovum on the anterior lobe of the hypophysis.

5. The great changes in the production of folliculin and prolan following on the fertilization of the ovum are neither conditional to nor consequent upon every fertilization, since they do not occur as a general rule but only in certain animals. The mass excretion of œstrogenic hormone which has hitherto been determined only in association with a fertilized ovum can be found also in male animals of the equine species. The testes of the horse contain much more œstrogenic hormone than does the placenta.

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PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

January 20, 1934.

The relation between the monophasic and the diphasic electrical response. By A. V. HILL.

Assume that a wave of electrical potential, $y=f(t-x/v)$, passes along a conducting fibre at constant speed v and without change of form: y is potential, t is time, x is distance. The true monophasic response is then $y=f(t)$. The difference of potential between two uninjured points, x_1 and x_2, is then $$y_1-y_2=f(t-x_1/v)-f(t-x_2/v).$$

(*a*) *Distance between electrodes short.* Assume that $(x_1-x_2)=l$ is short compared with the dimensions of the wave itself; the diphasic response is then $$y_1-y_2=\frac{l}{v}f'(t).$$

Thus the size of the diphasic response is proportional to the time of transmission ($\theta=l/v$) of the wave over the distance between the electrodes, but its form is independent of that time and distance. Since it is easy and accurate, by numerical integration, to calculate the form of the curve $y=f(t)$ from that of $Y=f'(t)$, the monophasic response can be deduced from the diphasic one provided that the distance between the electrodes is not too great. It is unnecessary, therefore, to injure a tissue to obtain the true form (not the size) of the monophasic response. All that is needed is a good diphasic record and a piece of squared paper.

(*b*) *Distance between electrodes finite.* It is easy, by successive subtraction, to calculate the diphasic response Y from the monophasic response y, for any given time θ of transmission (short or long) between the electrodes. It is simply $$Y=f(t)-f(t-\theta).$$

Conversely, given θ, the monophasic response can be calculated from the diphasic by successive addition. Up to $t=\theta$, $f(t-\theta)=0$ and $y=f(t)=Y$. From $t=\theta$ to $t=2\theta$, $y=f(t)=Y+f(t-\theta)$: Y is known and $f(t-\theta)$ has been determined up to $t=2\theta$, so y can be calculated. Similarly, from $t=2\theta$ to $t=3\theta$, etc. Without a knowledge, however, of the time θ the calculation cannot be made.

(*c*) *The velocity of transmission.* With a short distance between the electrodes the time θ and the speed v cannot be determined from the diphasic response. With a great distance (such that of $f(t)$ and $f(t-\theta)$ at least one must always be zero) they obviously can. For intermediate distances θ can be determined approximately, with a smaller error the greater the distance, as follows: assume a value for θ, calculate and plot the monophasic response from the diphasic as in (*b*), and repeat the process, with different assumed values of θ, until a smooth and reasonable form of the monophasic response is obtained.

Microscopical specimens of the heart.

By A. F. Stanley Kent.

Some phenomena in the behaviour of the heart can best be explained as due to the presence of definite structures. Such for instance is the persistence of a coordinated rhythm of the chambers after destruction of known channels of communication.

The demonstration of such a structure may, however, be difficult and tedious.

The specimens shown illustrate conditions existing in a bridge of tissue one millimetre wide which formed the sole connection between the two chambers of the right heart in an animal where a coordinated rhythm persisted. The character of the conducting path may be seen.

Slide 1. Origin of strand of conducting tissue from auricular muscle.

Slide 2. Course of strand in connective tissue of A.-V. groove.

Slide 3. Connection of strand with ventricular muscle.

The facilitation of the adrenaline response to histamine by carbon dioxide.

By H. A. Dunlop. (*Department of Physiology, King's College, London.*)

In some cats, as shown by Burn and Dale, the injection of histamine calls forth a secretion of adrenaline. In other cats under chloralose and especially in winter months, this secretion cannot be demonstrated. If, however, 5–10 p.c. carbon dioxide is administered, it is found that the injection of histamine causes a secretion of adrenaline, as shown by blood-pressure and plethysmographic experiments.

Human electrocardiograms. By BRYAN H. C. MATTHEWS.

The human electrocardiogram recorded with the cathode ray oscillograph has been the subject of numerous papers by Rijlant [1931, 1932*a*, *b*]. In these papers the presence of large slow waves (S_2, T_2) is reported. Prof. Rijlant has kindly given me permission to reproduce some of his figures; Fig. 1 A, B are typical examples of these, showing S_2 waves many times the amplitude of the P wave, and T_2 waves about as large as the T wave.

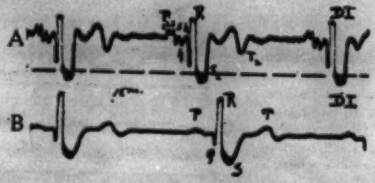

Fig. 1. Electrocardiograms taken by Rijlant [1932] with the cathode ray oscillograph. A, Amplification 400,000. B, 100,000.

In May I published [1933] records taken with a cathode ray oscillograph and a battery-coupled amplifier in which these large waves were not apparent; the records were essentially similar to the classical electrocardiogram recorded with the string galvanometer. I suggested that the large slow waves (many times the amplitude of the P wave) were a result of amplifier distortion rather than physiological activity. Rijlant has now adopted a battery-coupled amplifier [1933 *a*], and the records he obtains with it agree with mine in not showing the large slow waves. Fig. 2 A, B, C, D are reproductions of his records.

In the records which I criticized the amplitude of the S_2 waves was five to eight times that of the P waves (as the records were not calibrated and the R waves admittedly distorted, the P wave is the only standard of comparison possible); the mean area of the S_2 waves was about twenty-five times that of the P waves. Fig. 2 A, D shows that with Rijlant's present amplifiers the S_2 and T_2 waves have vanished from many of his records, in others the S_2 wave is barely as large as the P wave and may be of either sign; had S_2 waves twenty-five times the area of the P waves, or T_2 waves equal to the T wave occurred they should be very apparent in these records.

Rijlant suggests [1933 *b*] that I did not find S_2 and T_2 waves in my records owing to inadequate amplification; I have since taken records both with the cathode ray oscillograph and with my own, using ampli-

fication as high or higher than that used by Rijlant, and am still unable to find either the large S_2 and T_2 waves originally reported by him or any consistent deflection following the S wave. A typical record of mine is shown in Fig. 2 E, my records agree with Rijlant's (Fig. 2 A–D) in that

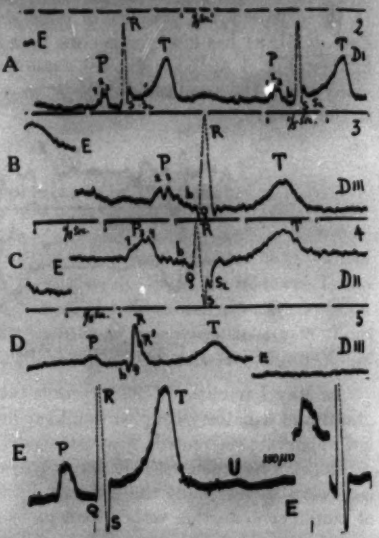

Fig. 2. Electrocardiograms taken by Rijlant [1933 *a*]. All from different subjects. A, D, Calibration at E 0·4 mv. B, Calibration at E 0·3 mv. C, Calibration at E unstated. E, Electrocardiogram taken in Cambridge with a moving-iron oscillograph and four-stage direct-coupled amplifier. Calibration at E 0·15 mv.

after the S wave with some subjects the curve is slightly above the base line, in others below (if we take this as an S_2 wave its mean value is nothing), that there is never an S_2 wave twenty-five times (or even comparable to) the P wave in area, and that T_2 waves do not occur with normal leads.

Since Rijlant's records [1933 *a*] and my own now seem to be in substantial agreement, it is difficult to avoid the conclusion that the very large S_2 and T_2 waves which he originally reported [1931, 1932] were a product of amplifier distortion as was the form of the R waves in his earlier records, which he pointed out at the time were greatly distorted by overloading of the valves in his amplifier.

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A right auricular pressor reflex. By R. J. S. McDowall.
(*Department of Physiology, King's College, London.*)

The fact that section of the vagi under certain conditions causes a fall of arterial pressure led to the description of a vago-pressor reflex by the author in 1924. The view was later supported by Anrep and Starling, but hitherto it has only been possible to demonstrate the reflex in conditions of low arterial or venous pressures. Evidence is now put forward which suggests that pressor impulses arise normally in the right auricle. The new facts are as follows: (1) Painting the right auricle with cocaine has the same effect as, and abolishes the effect of, section of the vagi in conditions when the vago-pressor reflex is evident. (2) The raising of the venous pressure by the injection of saline stimulates the vaso-motor centre as shown by constriction of perfused vessels of the hind limbs in functional connection with the rest of the animal by nerves only. (3) The raising of the venous pressure has been found in some cats to reduce or even abolish the normal depressor effects of impulses from the carotid sinus in the intact animal.

It is therefore suggested that there exists in the right side of the heart a cardio-vascular reflex which is not only accelerator, as shown by Bainbridge, but also pressor, analogous to the inhibitory-depressor reflex of the left side. Experiments show however that the afferent pathway is not confined to the vagus. A predominance of the accelerator-pressor reflex over the inhibiting depressor reflex would permit the rise of arterial pressure which occurs in exercise.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

February 17, 1934.

The innervation of the œsophagus and cardiac sphincter.

By G. C. Knight. (Introduced by H. Hartridge.)

This work has been undertaken with the object of examining previous theories and, if possible, to extend our knowledge of this subject.

With regard to the *cardiac sphincter*, three theories have been held:

1. That there is a true sphincter, containing smooth muscle which varies in tone from time to time.

2. That there is no true sphincter, but that the cruciate fibres of the diaphragm act in this capacity.

3. That there is a fold of mucous membrane which acts like a valve.

My findings support the first of these theories. The results obtained from electrical stimulation of extrinsic nerves in cats were as follows:

Stimulation of right vagus in neck caused relaxation of the cardiac sphincter.

Stimulation of fibres derived from the cœliac ganglion caused contraction of the cardiac sphincter.

Evidence for the existence of an intrinsic sphincter at the cardia is that relaxation occurs on vagal stimulation and contraction occurs on sympathetic stimulation. These results can still be obtained with the diaphragm completely divided.

With regard to the *œsophagus*, previous work by Ken Kure [1929*a*] suggests that in this organ the vagus and sympathetic exert the same action.

My findings do not support this theory. I find that stimulation of the vagus causes increased tonus and motility in the middle third of the œsophagus, whereas sympathetic stimulation produces the reverse effect.

Effect produced upon the œsophagus and cardiac sphincter, by section of the extrinsic nerves of supply. Previous work by Ken Kure [1929*b*] showed that section of the vagi resulted in a paralysed dilated œsophagus, and a contracted cardiac sphincter, and that section of the sympathetic fibres passing to the sphincter increased the obstruction which resulted at this region from bilateral vagectomy.

My results do not entirely support this view. The results obtained were as follows: Whereas bilateral vagectomy caused a paralysed dilated œsophagus and contracted sphincter, as found by Ken Kure, bilateral thoracic sympathectomy was followed by hyperactive peristalsis and a relaxed sphincter.

The above sections combined in the same animal caused a dilated œsophagus and diminished sphincter tonus, whereas Ken Kure found increased tonus at this region.

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The nature of the pulmonary alveolar epithelium. By F. HAYNES. (*Medical College, St Bartholomew's Hospital.*) (Introduced by H. HARTRIDGE.)

There are three views of the nature of the pulmonary alveolar epithelium: (i) that it is composed of nucleated cubical cells lying among non-nucleated squamous cells; (ii) that the squames are also nucleated, though the nuclei are only seen with difficulty; and (iii) that the so-called squames are merely wing-like processes of the cubical cells.

Fœtal and adult lungs of the cat, rabbit, rat and mouse, adult lungs of the monkey, sheep, ox, dog, horse (over 60 specimens), pneumoconiotic colliers (10 cases), normal and experimental guinea-pigs (several hundred specimens) and sections of lung from six months' consecutive post-mortem examinations at this Hospital have been examined. Guinea-pig lungs were examined: (i) collapsed; (ii) strongly distended; (iii) after silvering; (iv) after maceration with alcohol, chromic acid, acetic acid and potash; and (v) after exposure of the animals to nearly pure oxygen for 48 hours.

In no instance has a non-nucleated cell, either free in an alveolus or bronchus or forming part of the alveolar wall, been identified.

It is suggested that the normal pulmonary alveolar epithelium consists entirely of flattened nucleated cells, any of which has the power of becoming cubical or spheroidal on ingesting inhaled or other particles, and of becoming free as a "dust-cell" or "heart-failure cell" either in the alveoli or in the lymphatics.

Synaptic transmission through a sympathetic ganglion.

By J. C. Eccles. (*Physiological Laboratory, Oxford.*)

A previous communication [Eccles, 1933] contained a preliminary account of the complex action potentials which a preganglionic volley sets up in the superior cervical ganglion after a short synaptic delay. By using minimal stimuli it can be shown that this complex potential is made up of a standard type of electrical response having three components, and presumably each neurone gives a response of this form. A negative "spike" 6–8σ in duration continues on to a slow negative wave which probably lasts for at least 100σ, its actual end being indeterminate on account of the overlapping slow positive wave which continues for at least 0·5 sec. after the stimulus. The temporal dispersion of the responses of the individual neurones would account for the complexity of the initial part ("spike") of the integrated potential change.

The action potential of the postganglionic nerve trunk resembles the compound "spike" part of the ganglionic action potential, but is delayed by an amount which corresponds with the known rate of conduction in postganglionic fibres, hence it may be concluded that the "spike" part of the ganglionic action potential is caused by the setting up of impulses in the neurones of the ganglion, and these impulses travel directly along the postganglionic axons. The slow negative and positive waves are not present in the postganglionic action potential.

An antidromic volley backfired into the ganglion by a stimulus applied to the postganglionic trunk (internal carotid nerve) gives rise to the compound "spike" part of the ganglionic action potential after a latent period which appears to be entirely due to conduction time. An antidromic volley gives rise to neither the slow negative nor positive waves, nor does it affect the slow negative or positive waves produced by a preganglionic volley.

The "spike" produced by a submaximal preganglionic volley may be increased by a preceding maximal or submaximal volley set up by a stimulus applied through the same or other electrodes. This increased response is always present at intervals from 10 to 100σ after the first stimulus, and it attains a maximum at about 15σ, the greatest effect being observed when both volleys are small and equal. Similar results are obtained when the postganglionic action potentials are recorded, indicating that there has been a corresponding increase in the number of neurones discharging impulses, *i.e.* the first volley has facilitated the response to the second in exactly the same way as occurs with spinal reflexes. This facilitation in the ganglion indicates that submaximal

preganglionic volleys produce a "subliminal fringe" of excited neurones in which the intensity of the central excitatory state, C.E.S., is insufficient to set up a discharge. The response to a maximal preganglionic volley is not increased by a preceding maximal volley, hence a maximal volley must set up a discharge in all the neurones. There must therefore be an overlapping distribution of preganglionic fibres on to the postganglionic neurones, for otherwise the subliminal fringe produced by submaximal volleys would be present with maximal and would give facilitation. The facilitation of the response to a maximal volley is, however, indicated by the shortening of its synaptic delay [Eccles, 1933]. The curve expressing the time course of facilitation corresponds closely with the slow negative wave of the ganglionic action potential.

Painting the ganglion with dilute (0·01 p.c.) nicotine abolishes both facilitation and the slow negative and positive waves, but does not affect the "spike" recorded either from the ganglion or postganglionically. Facilitation and the slow waves return together as the nicotine effect passes off. Larger doses of nicotine, *e.g.* 0·02 p.c., reduce or abolish the "spike" both in the ganglion and postganglionically, *i.e.* conduction through the ganglion is blocked. The "spike" set up by an antidromic volley backfired into a ganglion appears to be abolished earlier than that set up by a preganglionic volley. Eserine in moderate doses has no effect on either the potential waves in the ganglion or the facilitation curve. In large doses it reduces or abolishes the ganglionic action potential. The liberation of acetyl choline in an excited ganglion [Feldberg and Gaddum, 1933] is therefore not responsible for the transmission of impulses through the ganglion.

Experiments with maximal and submaximal preganglionic volleys at various intervals indicate that the slow negative wave and facilitation are produced by a preganglionic volley both in neurones which discharge an impulse and in those which lie in the subliminal fringe. Since the facilitation curve is similar in time relations to the slow negative wave, and since both are similarly affected by nicotine, it appears likely that the slow negative wave is the potential sign of the C.E.S. which is responsible for the facilitation [Gasser and Graham, 1933]. The slow positive wave does not appear to be associated with any changes in the excitability of the ganglion.

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The effect of adenylic acid and adenosine on the human heart and blood vessels. By F. ALAN RICHARDS.

Embden isolated adenylic acid from muscle in 1927. Two years later Drury and Szent-Gyorgyi found the same substance to be the active principle of an extract of muscle having a peculiar action on the guinea-pig's heart, namely, sinus bradycardia and heart block. Adenosine had a similar action. The same effect was produced in other animals, together with dilatation of the coronary arteries and peripheral blood vessels. It seemed desirable to know whether man responds to an intravenous injection of these substances in the same way as the guinea-pig, rabbit, cat, dog and monkey.

The effects of these substances on the human heart, blood-pressure and skin temperature were studied. Yeast adenylic acid, or adenosine, or, in a few experiments, muscle adenylic acid, was injected in slightly alkaline solution into subjects of various ages and either sex, in all of whom the heart beat was regular and none of whom had gross heart failure. A record of the heart's action was made in many experiments, either by polygraph or continuous electrocardiograph. The blood-pressure was taken repeatedly before, during and after the injection. In the few experiments that have been made on the skin temperature, thermo-couples were placed usually on the cheek and leg and connected alternately to a galvanometer.

The effects of injection were not the same in every subject. The variations are perhaps due to the subjects' emotional responses to the injection and to the succeeding symptoms. The effects were these:

1. The blood-pressure was unaffected in most experiments. Man differs in this particular from the rabbit, cat, dog and monkey.

2. The heart invariably beat more quickly. In animals slowing is produced. There was in many subjects a transient slowing at some period, less marked than the quickening. The auricular and ventricular complexes were unchanged except in one subject, a man of sixty, whose *P* wave became inverted for 40 sec.

3. In about three-quarters of the subjects whose heart's action was recorded the conducting tissue was apparently unaffected. In the remaining quarter conduction was interfered with, sometimes to the extent of block.

4. The temperature of the cheek rose in five out of eight experiments and was unchanged in three. The temperature of the leg or finger was unchanged in five out of seven experiments; it rose and fell respectively in the other two.

All three compounds (yeast and muscle adenylic acids, and adenosine) produced these effects. In their effects on the pacemaker and conducting tissue the two adenylic acids were equally active. The tachycardia following their injection was of longer duration than that following injection of adenosine.

Ground congo-red fibrin for testing velocities of enzymes. By H. HARTRIDGE.

For class purposes congo-red fibrin, milk, and starch solution, have been in use for testing respectively the proteolytic, lipolytic, and amylolytic velocities of solutions of pancreatic juice. In recent samples of the juice the proteolytic test has required a considerably more concentrated solution than that required by the other two tests, in order that they should all take about the same time. In order to increase the proteolytic velocity, ground fibrin has been used in preference to fibrin shreds, which had been previously used. The ground fibrin is prepared very simply as follows: 50 g. of fibrin, which has been washed in water until the washings are colourless, is minced and placed in 100 c.c. of 0·5 p.c. congo-red solution for 24 hours. The fibrin is now placed in excess water, heated to 80° C. for 5 min., and is collected on a cloth and washed with water until the washings are colourless. It is then squeezed as dry as possible, placed first in alcohol, then in ether and removed to a desiccator until it is dry. It is now ground as finely as possible in a mortar and stored for use. When testing the activity of a proteolytic solution a pinch of the fibrin powder is placed in it and the colour estimated at known times in the usual way.

Vitamin B_1 and the kidney. By R. H. S. THOMPSON.
(*Department of Biochemistry, Oxford.*)

In order to settle the question as to whether the respiration of tissues other than the brain in the polyneuritic pigeon is affected by vitamin B_1 deficiency, experiments with and without the addition of crystalline vitamin B_1 have been made on the respiration of various tissues by a technique similar to those on the brain [Gavrilescu, Meiklejohn, Passmore and Peters, 1932]. No effects were observed in the case of muscle, heart or liver, but the kidney showed a striking difference in each experiment.

REFERENCE.

Gavrilescu, Meiklejohn, Passmore and Peters (1932). *Proc. Roy. Soc.* B, 110, 431.

Vacuum operated reaction velocity apparatus (stationary fluid method). By H. HARTRIDGE.

The reaction velocity apparatus of Hartridge and Roughton can be operated either by applying pressure to the fluids which are to react, or by applying suction to the mixture of the fluids after the reaction is over. For many purposes the ordinary water-driven vacuum filter pump is a convenient device when using the suction method. The suction pipe of the vacuum pump may either be attached directly by wide-bore tubing with the exit end of the observation tube, or a receptacle of suitable capacity may be evacuated prior to the experiment and the mixed fluids from the apparatus discharged into it. In addition to its application to the moving fluid type of reaction velocity apparatus the vacuum method also finds application to the stationary fluid apparatus.

An apparatus recently constructed consists of a T-shaped glass three-way tap, the two upper limbs of which are connected by rubber tubing to two glass tubes placed in vessels containing the two fluids which are to react together. The lower limb of the three-way tap is connected to a small glass cell. Where an optical method of analysis is to be employed, this cell has flat plates of glass forming its front and rear faces through which observation may be made. To the lower part of the cell is attached an ordinary single-way glass tap, the lower end of which is connected by a rubber tube to the vacuum pump. This apparatus is used as follows: the upper tap having been turned off and the lower tap having been turned on, the cell is evacuated. The lower tap is shut. The upper tap is now quickly turned so that both the reacting fluids are caused to enter the cell simultaneously until the cell is full. As the reaction proceeds suitable measurements are made from time to time. The lower tap is now opened and the cell emptied in preparation for a second charge. Where the reaction is a slow one (reaction half completed in not less than 30 sec.) a total time of filling of 1 sec. or less would probably be found satisfactory and an ordinary tap of 1 mm. bore may be used. Where quicker reactions are being followed and more rapid filling is required a tap of larger bore should be used. In either case tests should be made to prove that mixing is complete when the cell has just been completely filled. If this should be found not to be the case, mixing may be facilitated by giving the fluids a rotary motion on their way to the cell as in the other reaction velocity apparatus. A convenient way of obtaining this rotary motion is to have the plug of the three-way tap drilled with holes which are offset in respect of one another. The right-

hand hole is drilled inclined slightly towards the handle of the tap. The left-hand hole is drilled slightly inclined towards the other end of the tap. These two inclined holes join the hole in the plug down which the mixed fluids pass on their way to the cell. Since the fluids enter this in an approximately tangential manner, the fluids suffer a rotation on their way to the cell and are thus intimately mixed. A small dilatation or bulb of 3 or 4 mm. diameter in the bore of the tube between the three-way tap and the cell would appear to facilitate still further the rapidity of mixing.

Rotating disc method of changing phase in a musical tone.
By H. Hartridge.

Some years ago I described a method of introducing a sudden change of phase of π (a half wave-length) in a continuous single musical tone. The method used was to rotate suddenly through a few degrees the wind chest of a "de la Tours" syren during the rotation of the syren disc. Each time this was done the intensity of the tone perceived by the ears suffered a sudden diminution. This fall in intensity is readily explained on the resonance hypothesis, since the change in phase of the musical tone has caused it to be out of step with the ear resonators, so that the latter are first arrested and then recommence their vibrations. This experiment is quoted by Beatty. He adds, "Békesy, however, repeated the experiment with a different technique and was unable to detect any silent period." I thought it advisable therefore to repeat the original experiment in a different manner, and this was done as follows: A brass disc about 12 in. in diameter was turned truly on a steel shaft. An inner row of ninety-six equidistant slots was now milled through it from side to side, the distance between the first slot and the ninety-sixth slot being the same as the distance between any other two neighbouring slots. An outer row of ninety-six equidistant slots was similarly milled through it, the distance between the first and the ninety-sixth slot being only half that between any of the other slots, thus introducing a change of phase of π. When the disc is rotated by an electric motor and a stream of air is directed on to the inner row of slots, a continuous musical tone of constant pitch is produced. When the stream of air is directed on to the outer row of slots, a musical tone of constant pitch is heard, but this tone is interrupted once at each revolution of the disc when the change of phase in the tone occurs. Since the interruption effect heard

by the ears can be imitated by stopping up two neighbouring slots of the inner row, I conclude that the effect heard by the ears is a true interruption and not, for example, a change of pitch of the tone. This experiment, therefore, confirms in every respect my earlier experiment.

The apparent advance and retreat of black, white and colours.

By H. Hartridge.

It is well known that when blue and red objects are viewed side by side in the same plane the red objects appear to most people to lie considerably in front of the blue ones. When green or yellow objects are tested they are found to occupy an intermediate position. I have discussed the cause of the phenomenon in a previous paper under the heading "Chromatic Stereoscopy."

I have recently noticed that the phenomenon is not limited to coloured objects, for I find that it is possible for black and white to be advancing and retiring colours when placed on backgrounds of suitable colour. Thus when they are placed on a red ground, black advances and white retires; but when they are placed on a blue ground, white advances and black retires. These relative positions may be explained as follows: Considering the case of the red ground, a black object on a red ground is seen in the same position as a red object on a black ground would be. A white object on a red ground is, on the other hand, equivalent to a blue-green object on a black ground plus a uniform red ground without any object on it (as may readily be seen by merely summing the colours). Now a blue-green object on a black ground retires relatively to a red object on a black ground and, therefore, substituting the equivalents we have just found, a white object on a red ground retires but a black object on a red ground advances. Observation shows that a white object on a blue ground advances, whereas a black object on a blue ground retires, but a white object on a blue ground may be replaced by a yellow object on a black ground plus a uniform blue ground. We know, however, that a yellow object on a black ground advances, whereas a blue object on a black ground retires. Therefore we have explained the phenomenon in this case also. For grounds of intermediate colour between red and blue, namely yellow and green, the phenomenon is not so striking as it is for red or blue.

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The resonance theory of hearing. By H. HARTRIDGE.
(*Medical College, St Bartholomew's Hospital.*)

Recent work by Wever, Bray, Adrian, Hallpike and others on the transmission of musical tones by the auditory nerve has seemed to present difficulty to the resonance theory. Such incompatability as exists is more apparent than real however. The position may be briefly summarized as follows: According to the resonance theory when a musical tone of fixed pitch is transmitted to the cochlea it sets certain structures into vibration, and in consequence suitable sensory nerve endings receive stimulation. This stimulation is not continuous but periodic, since it occurs only during a particular phase of the movements of the resonators. Thus, suppose the resonators to be the fibres of the basilar membrane and the hair cells of Corti to be the end organs, the latter receive stimulation during one phase of the movement of the former. It might be (*a*) the ascending phase, or (*b*) the descending phase, or (*c*) the phase of upward acceleration, or (*d*) the phase of downward acceleration during which the stimulus to the hair cells is applied. Now suppose the fibres of the auditory nerve to be capable of transmitting N separate nerve impulses per second (where N is about 1000 and is called the "critical" frequency). Suppose a musical tone of a frequency below this to be falling on the ear, the resonators which correspond to it in frequency will be set into vibration and their hair cells will be stimulated a corresponding number of times per second. Thus a musical tone of 200 vibrations per second would bring about stimulation of the appropriate hair cells at the same number of times per second. At each one of these stimulations nerve impulses will be sent up the fibres of the auditory nerve, and since we have already made the assumption that the auditory nerve can transmit a greater number of separate impulses than this, 200 separate trains of impulses will be sent up to the brain. We may assume that the brain is able to identify the pitch of the sound in either one, or a combination of the following ways:

1. By a recognition of the fact that an isolated bundle of nerve fibres is transmitting impulses, and an appreciation of the identity of that bundle.

2. By a recognition of the frequency at which the trains of nerve impulses are arriving.

If a suitable electric recording apparatus be applied to the nerve it should be possible to identify these trains of impulses on their way to the brain. If the frequency of a musical tone falling on the ear be above the critical frequency then the impulses sent into the nerve fibres by the

hair cells will not be separately transmitted with periods of rest in between, and the refractory period of each nerve fibre will now modify the impulses probably in the following manner. Consider one resonating fibre with its hair cells and its nerve fibres. At a certain time interval after the commencement of the musical tone the amplitude of vibration of this resonating element will have become sufficiently large that its hair cells are adequately stimulated and thus commence to send nerve impulses into their nerve fibres. The first impulse starts up the nerves. The second impulse, since it arrives at a rate above the critical rate, falls within the refractory period of the nerve fibres and is obliterated. Subsequent impulses will be similarly dealt with until the refractory phase is over, when once more an impulse is accepted and transmitted and another refractory phase commences as before. Similar events are taking place in the other resonating elements which are set into vibration by a musical tone. Where the frequency does not greatly exceed the critical frequency it may be possible for the brain (and also good electric recording apparatus) to identify correctly the frequency of the incoming sound, since some nerve fibres will probably be in a condition to conduct impulses which others are obliterating. At frequencies considerably above the critical frequency it would seem that this substitution must fail however. This failure being due to slight variations in the velocities of nerve transmission in the different nerve fibres which are conveying impulses. An amplifier close to the ear might be able to detect the frequency correctly, but as points further from the ear were examined greater and still greater difficulty would be met with, so that the brain would have but one clue left for identifying frequency, namely, the appreciation of the identity of the bundle of nerve fibres connected to the resonators which are in vibration.

Further evidence against the theory of duodenal regurgitation. A statistical analysis of 389 fractional test meals. By N. F. Maclagan. (*Courtauld Institute of Biochemistry.*) (Introduced by E. C. Dodds.)

An attempt has been made to test the influence of pyloric function upon gastric acidity by an analysis of 454 gruel fractional test meals performed in the Courtauld Institute of Biochemistry, Middlesex Hospital, during the years 1931–3. Cases of pyloric stenoses and gross gastric deformity were excluded, leaving 389 cases, which were divided into the following classes: anacid (74), late secretion (13), rising (58), low plateau (76), peak (102), high plateau (21), climbing (45), and "bile

in more than two specimens" (61). In the high plateau and climbing curves the free acidity exceeded 45 c.c. *N*/10 HCl p.c.

The assumptions were made that the average emptying time and volume of residue at 2 hours are an index of the tone of the pyloric sphincter, and that the incidence of bile (expressed as a percentage of specimens containing bile) is an index of duodenal regurgitation. The sampling errors of these statistics were then calculated by standard methods with the following results:

(A) There were very few significant differences between the various types, except that the anacid curves showed a rapid emptying time and a small residue.

(B) There was no evidence of increased duodenal regurgitation in the peak curves. Moreover the fall of acidity corresponded to the appearance of bile in only 19 out of the 102 cases in this group.

(C) Curves containing a high proportion of bile did not show any difference from those in which bile was completely absent, either in type incidence or in motility.

(D) There was no evidence of pylorospasm or of failure of regurgitation in the curves exhibiting hyperacidity.

These results fail to give any support to the view that gastric acidity in man is controlled either by duodenal regurgitation [Boldyreff, 1915; Bolton and Goodheart, 1922] or by pylorospasm [Bolton and Goodheart, 1922]. They are therefore in favour of the alternative theories which explain hyperacidity by hypersecretion, and the "normal" (peak) curve by the secretion of a neutral [MacLean and Griffiths, 1928] or alkaline [Baird, Campbell and Hern, 1924] fluid, perhaps from the pyloric region [Baird, Campbell and Hern, 1924], perhaps by the mucoid glands of the stomach [Babkin, 1929].

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Apparatus for the stereophonic reproduction of sounds.

By H. Hartridge.

It is common knowledge that great advance has recently been made in the technique of sound reproduction such as is used in broadcasting. Sounds are now reproduced with fidelity covering nearly the whole of

the acoustic spectrum. In one respect, however, little or no advance has been made, namely, in reproducing the position of the sources of sound in space. Whereas the stereoscope gives a faithful reproduction of the positions of objects to the eye there is at present no corresponding instrument or "stereophone" to give objects their correct positions to the ear.

One method of stereophonic reproduction has been tried by Harvey Fletcher. Two microphones, in imitation of the ears, are placed on opposite sides of a dummy's head. By means of separate connections, amplifiers, batteries, etc., these sounds are reproduced at headphones, the right-hand microphone being connected to the right-hand headphone and *vice versa*. This apparatus works as follows: A source of sound in front of (or behind) the observer emits sound waves which, affecting the two microphones simultaneously, affect also the two headphones simultaneously, and the source of sound appears to be situated in front of (or behind) the observer. The sound wave emitted by a source of sound on the right-hand side arrives at the right-hand microphone in advance of the left-hand one, and so the right-hand headphone reproduces in advance of the left-hand headphone and thus the observer identifies the source of sound as appearing to lie on his right-hand side. He similarly identifies sounds from the left. The disadvantage of this arrangement is that headphones alone can be used, and it is not possible to reproduce the sounds by means of loud-speakers. An alternative arrangement which avoids this disadvantage may be briefly described as follows: The sources of sounds to be reproduced are placed in several sound-proof rooms, each of which contains a microphone. Each microphone is suitably connected to a loud-speaker. The loud-speakers are arranged in the same positions that the rooms are arranged.

An example may make the use of this method a little more evident. If a violinist is being accompanied on a piano each instrument would be placed in a sound-proof room provided with a microphone. Through glass partitions the performers would see one another and they would be able to hear one another by means of headphones. The listener would have two loud-speakers, one connected with the microphone in one room, and the other with that in the second room. He would thus hear the sounds of the piano coming out of one loud-speaker, and the sounds of the violin coming out of the other loud-speaker. Other more elaborate arrangements are possible, with a corresponding improvement in the results obtained.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

March 17, 1934.

A simple graphic method of showing extensor tone in dial anæsthesia. By GRACE BRISCOE.

Since it has been found that the responses of peripheral nerve muscle preparations (with circulation intact) may vary with the general tonic condition of the intact limbs it is convenient to have a simple objective method of showing the presence of tone in the opposite innervated limb.

Exaggeration of extensor tone, resembling in outward appearance the extensor hyperactivity of a mild decerebrate rigidity, is found fairly frequently in dial anæsthesia. The quadriceps resists attempts to bend the knee, "shortening" and "lengthening" reactions and the "clasp knife" sensation may all be elicited. Sometimes the resistance to bending is more marked in the forelimbs than the hindlimbs.

The femoral and sciatic trunks are cut on one side, and then both legs are put up in a similar fashion, by a drill through the lower end of the femur. Movements of the legs are recorded by threads attached to the patellar tendons.

Both limbs are lifted into full extension by pressure under the heels and then the support is suddenly withdrawn. The denervated leg falls at once into a position of flexion. The intact leg falls as rapidly as the toneless side to about the half-way position and is then suddenly checked and remains in a partially extended position (1). A clonus sometimes develops in this position, but usually the leg sags down gradually to a more flexed position. If tone be well marked, this sagging may be slight, and considerable force will have to be used to bring the leg back to the fully flexed position. Once there it remains flexed, but repeating the passive extension and release will bring about another shortening reaction.

If tone be slight the leg may show a temporary check near the end of its fall, but even this forms a clear contrast with the unchecked fall of the denervated side (2*a*, 2*b*).

If ether be given until there is no feeling of resistance in the quadriceps, the records from the two sides are indistinguishable (2*a*, 2*e*).

The method may be used to illustrate the action of drugs (3*b*), and the result of decerebration (4*b*).

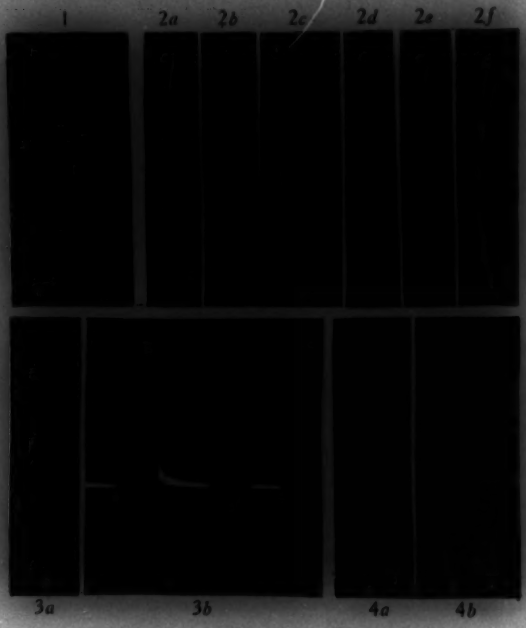

Fig. 1. Cat. Extension of knee is downwards.

Records on slow drum except 4 and small portion of 3*b*.

1. Dial anæsthesia, well-marked tone. From *A* to *B* the knee (nerve supply intact) is forcibly bent and at *B* released. The limb remains nearly flexed. From *C* to *D* the leg is carried into full extension and released at *D*. Its fall is checked half-way and a slight clonus develops.

2. Dial-ether. Slight extensor tone. (*a*) Fall of denervated and (*b*) fall of intact leg. Ether given. Preliminary accentuation of tone (*c*) followed by diminution (*d*) and finally loss of tone (*e*). (*f*) Return of tone after removal of ether.

3. Dial-ether. (*a*) Before strychnine; slight tone and tendency to clonus. (*b*) After 0·1 mg. strychnine nitrate intravenously. Tone increased, lengthening and shortening reactions with well-marked clonus. In less than a minute clonus disappeared and the muscle lengthened. Clonus reappeared as soon as leg released again from extended position.

4. Decerebrate rigidity. No dial. Preliminary ether. (*a*) Fall of denervated leg. (*b*) Fall of intact leg; shows that first part of fall is as rapid as on atonic side.

Direct chemical estimation of "carbamino-bound" CO_2 in hæmoglobin solution. By J. K. W. Ferguson and F. J. W. Roughton. (*Physiological Laboratory, Cambridge.*)

Henriques in 1928 revived the idea that not all of the chemically-bound CO_2 of the blood was present as bicarbonate, but that a part was also combined directly to the hæmoglobin, possibly in the form of a carbamino compound. The idea of a direct compound has since been supported by Margaria, and also by Meldrum and Roughton, who in addition brought forward definite evidence in favour of the carbamino hypothesis.

If the carbamino hypothesis is true, it might be possible to separate the carbamino-hæmoglobin compound from the bicarbonate of the blood by precipitating the latter with excess $BaCl_2$ at alkaline *p*H, as has been done so successfully by Faurholt in the case of the simpler carbamino compounds of ammonia, glycine, etc.

The difficulties met with in applying such a method to blood were:

(i) Formation of hæmatin at alkaline *p*H. This was avoided by using a very resistant species of blood, *i.e.* ox, and by cooling to 0° C. as soon as possible after the various reagents had been mixed.

(ii) Decomposition of the CO_2-Hb compound during the actual mixing of the blood with alkali, such decomposition being favoured by the large amount of carbonic anhydraxe present. This was avoided by mixing the alkali with the blood completely in less than 0·01 sec.

(iii) Slow decomposition of carbamino compounds might even occur after the mixing with alkali was complete. This was reduced to an average value of 7 p.c. by the same means as in (i).

(iv) The proteins of the blood might inhibit the complete precipitation of $BaCO_3$ by acting as protective colloids. This effect was found to be considerable in laked blood, but to be negligible in purified hæmoglobin solutions.

(v) The dissolved CO_2 of the blood might, when the blood is made alkaline, combine with the proteins of the blood to form carbamino compounds, and hence be estimated as such. This effect turned out to be quite appreciable, but could be allowed for by blank experiments.

In the final arrangement, three 5 c.c. syringes were filled respectively with (*a*) hæmoglobin solution equilibrated with various pressures of CO_2, (*b*) 0·05–0·20 *N* NaOH (according to the amount of CO_2 in (*a*)), (*c*) 15 p.c. barium chloride. The solutions were driven from the three syringes into a mixing chamber of the Hartridge-Roughton type (kindly loaned by

G. E. Millikan): the mixed fluid from the chamber was collected under paraffin in a centrifuge tube and promptly centrifuged for 15 min. at 0–4° C. A 5 c.c. sample of the supernatant fluid was then analysed for "unprecipitated" CO_2 in the van Slyke-Neill manometric apparatus.

With this method, and the controls already mentioned, small but appreciable amounts of unprecipitated CO_2 (and therefore presumably of carbamino CO_2) were found both at room temperature and at 38° C. Under comparable circumstances reduced hæmoglobin solutions invariably contained much more than oxyhæmoglobin solutions. This phenomenon was specially investigated in the physiological range, *i.e.* temp. 38° C., CO_2 pressures ranging from 35 to 65 mm. Hg., *p*H 7·2–7·6 roughly. Here it was found that, of the extra total CO_2 taken up by reduced hæmoglobin over that taken up by oxyhæmoglobin at the same CO_2 pressure, 50 p.c. or more might be attributed to difference in the carbamino content of the two solutions. This would seem to establish the importance of carbamino compounds in physiological CO_2 transport, even though the absolute amounts of such compounds in the blood may be small, *i.e.* not more than 5 p.c. of the total CO_2.

Pyruvic acid and the avitaminous brain. By R. A. Peters and R. H. S. Thompson. (*Biochemical Laboratory, Oxford.*)

Pyruvic acid is formed by avitaminous pigeon's brain (not by normal) during respiration *in vitro* in lactate solutions, and disappears largely upon addition of vitamin B_1. This is shown by nitroprusside reaction [Peters and Sinclair, 1934], and by present estimations of bisulphite binding substances [Clift and Cook, 1932] confirmed by a modified Neuberg-Case method. Added pyruvic acid behaves similarly. Increased respiration with pyruvate occurs regularly with added vitamin B_1 [contrast Meiklejohn *et al.*, 1932], in presence of pyrophosphate. These results are independent of added sulphite or fluoride and constitute a strong support to the view that pyruvic acid is a normal tissue metabolite [Meyerhof and Kiesling, 1933].

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Action potentials in the saccular nerve of the frog. By D. W. ASHCROFT[1] and C. S. HALLPIKE[2]. (*Ferens Institute of Otology, Middlesex Hospital, London, W.* 1.)

Since the publication of Magnus's monograph [1924] the evidence accumulated has failed to substantiate the view therein expressed that the saccule is an organ of so-called static equilibrium supplying to the higher centres information concerning the position of the head in space in relation to gravitational lines of force. Prior to this date Parker [1909] and Maxwell [1923] had failed to demonstrate any connection of the saccular macula with equilibrium in the dog-fish. Subsequently, McNally and Tait [1925] in the case of the frog and Versteegh [1927] in the case of the rabbit succeeded in carrying out extirpation experiments upon the saccular macula, employing operative methods of precision. In neither case did any significant disturbance of equilibrium result.

The present position regarding the function of the saccule is one of obscurity. In the investigation here recorded the problem has been approached upon electro-physiological lines. Employing McNally's technique for the micro-dissection of the frog's labyrinth it has been found possible to isolate the saccular nerve in lightly anæsthetized frogs, the cut end of the nerve being raised upon a micro-electrode of platinum controlled by a Leitz micro-manipulator. The entire preparation is then transferred *en bloc* to a platform upon which movements of rotation or tilting (fast or slow) can be carried out, the preparation being kept under observation through a binocular dissecting microscope. The active electrode, together with an indifferent lead from the frog's skin, are connected to the input of a high-gain resistance capacity coupled amplifier whose output passes to a cathode ray oscillograph or loud-speaker; suitable arrangements are made for the photographic recording of potential changes displayed upon the face of the oscillograph tube.

Results. No response could be elicited from the nerve by rotation or tilting movements. In response to vibration, however, marked reaction has in most cases been observed. This type of stimulus may be applied either in the form of light stamps upon the floor or the dropping of graded weights. Alternatively the stem of a vibrating tuning fork may be applied to the platform.

The response to the former variety of stimulus consists of a sharp burst of impulses, initially monophasic, which die away rapidly in frequency and amplitude.

[1] Baron Research Student.

[2] Working with a grant from the Medical Research Council.

The response to vibration from the stem of a tuning fork consists of a series of monophasic impulses in which stability of amplitude and frequency is quickly attained, the frequency corresponding exactly to that of the stimulating fork. With the 1024 ~ fork the response is definite but dies away rapidly. Owing to the high rate of decrement of this fork it is not possible to draw any conclusions either as to the neural significance of this rapid extinction of the response or as to the reproduction of frequency during the period of response.

It is not thought that the electrical changes shown in these records can be regarded as other than true action potentials set up in the saccular nerve endings by the vibrational stimuli described. This view is based upon the following considerations:

1. The electrode holding system and amplifier have been found sufficiently non-microphonic as to give a steady base line in the presence of vibrational disturbance of the order employed.

2. The strict localization of the potential changes to the saccular nerve, and the high degree of sensitivity displayed by the preparation to

(*a*) The short circuiting effect of excess of fluid in the operative field.

(*b*) Lack of sufficient fluid in the field; this leaves the saccular otolith high and dry from its normal fluid surroundings.

(*c*) Death of the animal.

The response under all three conditions outlined is speedily abolished.

3. Abolition of response by cocaine.

A drop of 2 p.c. cocaine is placed upon the operative field. Two minutes later this is sucked away by a micro-pipette, approximately optimal electrical conditions being restored. Under these circumstances a previously brisk response is found to be abolished.

Conclusions.

The saccule in the frog contains end organs concerned with vibrational sensibility. The propagated electrical changes reproduce accurately the frequency of the stimulus, at any frequency up to 512 ~. At 1024 ~ a response occurs, though evidence of frequency reproduction is unsatisfactory.

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The function of the tensor tympani muscle. By C. S. HALLPIKE[1] and A. F. RAWDON SMITH[1]. (*Ferens Institute of Otology, Middlesex Hospital, London, W.* 1.)

The early theories as to the function of this muscle have been adequately summarized by Köhler [1909], Kato [1913], Schaffer and Giesswein [1926] and Mangold [1926]. Later investigators, especially Kobrak [1930] and de Nó [1933] are agreed that reflex contraction of the muscle appears in response to acoustical stimulation. Still other investigators [Hughson and Crowe, 1933] have also noted the contraction in response to mechanical stimulation of the pinna. In contradistinction to other work, that of de Nó [1933] shows that the response may be elicited in favourable preparations by sounds of relatively low intensity. (Some 80 decibels above the human threshold over the middle range of frequencies.) This communication records the results of observations upon the reflex activity of the tensor tympani in cats.

In these experiments, cats under light nembutal anæsthesia, or in many cases decerebrate preparations, were used. In the latter the reflex is particularly brisk.

In this connection it is worthy of note that Hughson and Crowe [1933], in a series of 200 cats, failed altogether to demonstrate the phenomenon. It is likely that this failure may be explained as being attributable to the employment of too deep a degree of anæsthesia.

In our experiments we have employed as a source of sound a loud-speaker, activated *via* an amplifier by a heterodyne oscillator, the output being suitably controllable for intensity and frequency. Considerable attention has been devoted to the effect upon the Wever and Bray phenomenon of the reflex contractions of the tensor tympani. In this part of the work a resistance-capacity coupled amplifier, of conventional design, has been employed in conjunction with a cathode ray oscillograph. Photographic records are made upon fast moving Cine bromide paper. Direct observations of the contractions of the muscle were made in all cases through a binocular dissecting microscope.

We have observed brisk contractions in response to pure tones of frequency 200–2000 cycles, of intensity some 80 decibels above the human threshold. The contractions are particularly well marked within the range 500–1000 cycles. As far as may be judged by eye, these appear to take the

[1] Working with a grant from the Medical Research Council.

form of an initial twitch, followed by a smaller contraction maintained so long as the sound is applied, and a terminal twitch when the stimulus is removed. The reflex is entirely abolished in deep anæsthesia, and is never present in the large proportion of cats (some 40 p.c.) in which the external meatus is found to be filled with wax (cf., however, de Nó [1933]). Further, we doubt the theory that this is a tendon reflex, owing to the persistent presence of reflex contractions observed in the muscle, following upon divisions of its tendon.

It is possible to summarize the effect of these contractions upon the Wever and Bray phenomenon, using frequencies of 500–1000 cycles, as follows:

(1) On switching on the speaker, the Wever and Bray response rapidly attains full amplitude. The shortest interval observed is approximately 2σ.

(2) Following this, the response shows a gradual decrease, attaining a level some 5–6 decibels below the initial amplitude. It is noteworthy that the overtones, which are introduced by the vibrating components of the ear (these being absent in a microphone record), appear to suffer a greater relative decrement than does the fundamental.

(3) Following this period of decrement, the lowest level of which is attained some 30σ after its initiation, there follows a period approximately equal in time, during which the response rises. The initial value, however, is never regained. It may be seen, therefore, that these stages correspond in sequence to those of the contractions of the tensor tympani outlined above.

(4) On switching off the sound, the response disappears relatively slowly; that this is not altogether due to persistent vibration of the loudspeaker diaphragm has been demonstrated with the microphone.

On cutting the tensor tympani tendon, a fall in response of approximately 1–2 decibels takes place. This may possibly be due to the release of the tension normally exerted by the tone of the resting muscle and by its fascial sheath on the tympanic membrane, which, following upon this operation, immediately becomes somewhat flaccid. This operation removes all the effects detailed above under (2) and (3). The response is fully maintained, after its onset, but on switching off the response here shows a slower decrease in amplitude and considerable irregularity. This is indicative of the possible damping action of this muscle.

We conclude that reflex contractions of the tensor tympani exert a damping effect upon the amplitude of movement of the vibrating mechanism of the ear. The short duration and small degree of this

damping effect do not wholly favour the protective theory of the muscle's action. Overtones are relatively more affected by this damping action.

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The effect of ether on the action of adrenaline.

By Hugh A. Dunlop. (*King's College, London.*)

Since the work of McDonald, it is well recognized that ether anæsthesia is largely responsible for the depressor action of small doses of adrenaline. This action has now been studied by means of plethysmographic records of skinned and unskinned limbs in cats anæsthetized in the first instance with ether and subsequently with chloralose. In the skinned limb there is throughout an increase in volume with each dose of adrenaline, but before the ether has passed off only a slight diminution in the volume of the whole limb is obtained. Gradually, in the latter, the adrenaline constriction becomes greater and greater until when the same dose of adrenaline, which formerly caused a fall, now gives a rise of arterial blood-pressure, the constriction is very marked.

The results support the view previously put forward by Dunlop that whether adrenaline causes a rise or fall of blood-pressure depends chiefly on the amount of skin (and visceral) vaso-constriction which the drug is able to induce. This constriction tends to be abolished by ether.

An apparatus for the study of whole isolated arteries.

By F. S. Gorrill. (*King's College, London.*)

The apparatus consists essentially of a plethysmograph in which the artery may be placed and perfused with various solutions. The changes in volume show themselves in a horizontal capillary tube where the movements of the fluid may be photographed. The recording apparatus is made from an ordinary film camera driven by a gramophone motor. Results indicate that the artery can be caused to develop a tone which can be diminished or increased by appropriate treatment.

A special purpose amplifier. By BRYAN H. C. MATTHEWS. (*King's College, Cambridge.*)

The amplifier described below was designed for multiple recording, and has been in use for some months in a three-oscillograph recording system. It has been found to have several advantages over the conventional design of amplifier which make it specially suitable for use in the first stage of any amplifier for physiological purposes.

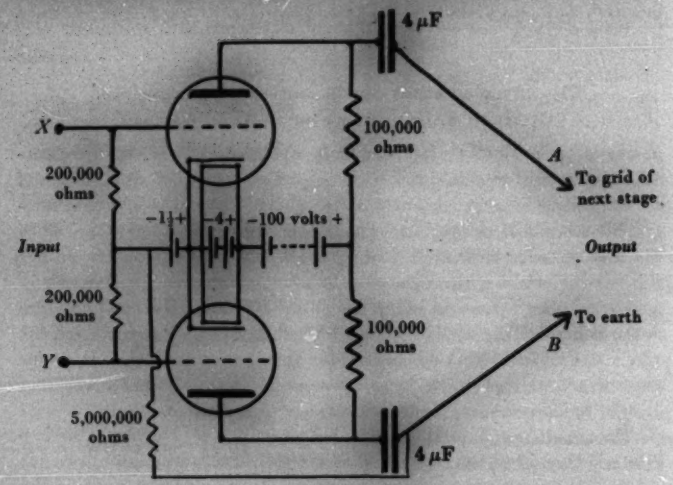

Fig. 1.

Its advantages are: (1) immunity from electrical feed back from later stages to the preparation; (2) great reduction in the stimulus escape when electrical stimulation is employed; (3) comparative freedom from external electrical interference; (4) no interconnection when several amplifiers are used simultaneously.

Fig. 1 shows the general arrangement of the first stage which may be followed by any conventional type of amplifier. At present Catkin valves (MH 4) are in use and are quite satisfactory, the pairs used must be roughly matched, the possibility of high frequency oscillation occurring can be eliminated by connecting a 0·001 μF condenser and a 5000 ohm resistance in series between each anode and cathode. As the batteries

are not at earth potential, the whole assembly including the batteries must be screened in an earthed case.

It will be seen that neither of the input leads goes to earth, so that the amplifier is only affected by potential changes between *X* and *Y*, and if the potential of both *X* and *Y* changes equally relative to earth there is no resultant change between *A* and *B*. This is the reason for (1) and (3) above, for most forms of electrical interference seem to result from electric fields fluctuating relative to earth potential; while such fields disturb an amplifier where the input goes to grid and earth by making the grid potential fluctuate, they affect both grids of this arrangement equally and so are not amplified.

If a pair of electrodes is placed on a frog's heart and a second pair on the liver, when these are connected to two recording systems having grid + earth input amplifiers, the pair on the liver yield an electrocardiogram only slightly smaller than that recorded from the pair on the heart, and of opposite sign; such potential changes in the liver being improbable, on further consideration it is clear that the effect is due to the interconnection of two of the electrodes *via* the common earth. Obviously very misleading results might follow from the simultaneous use of two grid + earth input amplifiers.

Using the amplifiers having grid + grid input circuits this anomaly disappears, the electrodes on the liver no longer show any substantial electrocardiogram, the deflections being reduced some thirty times from their former magnitude.

These amplifiers can thus give a true picture of changes between several points on the same preparation which is often unobtainable with grid + earth input amplifiers.

Impulses leaving the spinal cord by dorsal nerve roots.

By Bryan H. C. Matthews. (*King's College, Cambridge.*)

In the course of observations on the impulses in spinal nerve roots I have found that there is considerable activity in the central ends of cut dorsal rootlets. Recent histological work [*e.g.* Kahr and Sheenan, 1933] has established the presence of a small number of fibres in dorsal roots which do not degenerate centrally from a section between the dorsal root ganglia and the cord. It is thought possible that the activity here reported is that of these fibres.

Cats have been anæsthetized, and after lumbar laminectomy decerebrated and the anæsthetic discontinued. The electrical activity of

the intradural nerve rootlets is recorded with an amplifier and moving iron oscillograph. As yet only the 5th, 6th and 7th lumbar roots have been examined.

Even the smallest rootlets of the dorsal roots show evidence of the activity of very large numbers of sensory fibres, and analysis of the ascending impulses is only possible when the majority of the fibres have been severed distal to the recording electrodes. The results of this analysis will be published later.

If the rootlets are cut and electrodes placed on their central ends there is still considerable activity due to impulses leaving the cord by them. It is estimated that roughly 5 p.c. of the fibres in the dorsal roots are still active after ascending impulses have been cut off by section peripheral to the recording electrodes.

By partial section central to the recording electrodes I have been able to record the impulses leaving the cord in preparations of a single active fibre. Three types of discharge are present: (*a*) those in small fibres evidenced by slow action potentials; (*b*) discharges of motor neurones (large fibres); (*c*) antidromic sensory discharges (large and medium fibres).

The activity of the small fibres (*a*) has not yet been analysed.

(*b*) These discharges are attributed to motor neurones for the following reasons. (1) The rate of rhythmic discharge is never above 70 per sec. (2) The stimulus which evokes or modifies them is movement of or pressure on the limbs. The receptive area is wide in contradistinction to (*c*). They are often slowed or stopped by like stimulation of the opposite limb. Often stimulation of a forelimb controls the discharge in 6th or 7th lumbar roots. (3) Cutting all the other sensory rootlets of the same and adjacent roots may reduce but seldom abolishes the discharge occurring when the appropriate region is stimulated. (4) The rate of the discharge is in nearly all cases greatly influenced by the position of the head, the discharge can often be started and stopped by raising and lowering or rotating the head. (5) The discharges are often accelerated in the early stages of chloroform and ether anæsthesia and are abolished in the deeper stages. (6) The fibres concerned do not appear to be derived by branching from motor fibres leaving the cord by the corresponding ventral roots as cutting and stimulating these with crystals of salt does not affect the discharge.

(*c*) Antidromic sensory discharges. (1) These can be evoked by appropriate stimulation of some sharply defined area, either by movement of hairs, pressure, stretch of a muscle, or joint movement. (2) They can

occur at high frequencies, an initial frequency of 650 per sec. has been recorded from a superficial receptor on sudden application of pressure. (3) The response in any single fibre preparation is abolished by section of some particular rootlet entering the cord from the same or another root. (4) They are not abolished by deep anæsthesia and are often increased in frequency (this may be due to the effects of failing circulation on the nerve endings from which they arise). (5) Prolonged discharges never occur, the impulses always appear in bursts at a frequency depending on the intensity of sensory stimulation, with pauses of 0·2–0·8 sec. between. The discharges are like intermittent samples of a sensory response which are managing to get across a region where conduction fails periodically. No correlation has yet been found between the frequency and number of impulses in each burst and the length of the pause which follows. These impulses thus appear to come from sensory receptors, to enter the cord and leave it again by a different rootlet after passing through some region with properties unlike those of a nerve fibre.

No activity of any sort has yet been detected in the peripheral ends of cut ventral rootlets.

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The effect of grafting or removing the posterior limb-rudiment on the spinal cord of Amphibian embryos [*Discoglossus pictus* (Otth.)]. By Raoul M. May. (Introduced by J. Needham.) (*Collège de France and Institut Pasteur, Paris, France.*)

Braus [1906], Dürken [1916], as a result of ablations of the posterior limb-bud in Amphibian embryos, and Shorey [1909], after ablations of the wing-bud in the chick embryo, deduced that relations exist between the peripheral organs and the neuroblasts, both sensory and motor. Detwiler [1920, 1924], however, following his experiments of grafts and ablations of the forelimb rudiment in the Urodele *Amblystoma*, admitted only the sensory relations, and postulated that the motor neuroblasts were under the influence of internal factors of the neuraxis.

But I have shown [May, 1930], by means of grafts of the embryonic spinal cord, that the absence of the lumbo-sacral plexus, due either to the absence of a posterior limb whose place is occupied by a grafted cord, or to the fact that the limb is present but innervated by the implanted

cord, reverberates on the autochthonous spinal cord and its ganglia through a sensory and motor hypoplasia. It therefore follows that the hindlimb innervation plays a part in the development of both the sensory and motor neurones of the neuraxis. The present experiments confirm these results.

The rudiment of the right hindlimb was transplanted from an embryo of the Anuran, *Discoglossus pictus* Otth., at the tail-bud stage, on to the side of another embryo of the same egg lot, in the region immediately beneath the spinal cord. The grafted limb developed synchronously with the autochthonous hindlimbs, and contracted nervous relations with the cord.

The innervation of the grafted limb depended on the place of its implantation, and was not specific. In some cases it was innervated by nerves of the lumbo-sacral plexus, in others by nerves of the brachial plexus, and in others by nerves belonging to no plexus.

In all cases of nervous surcharge due to the innervation of a grafted limb, the cord, at the level of emission of the supplementary nerves, was asymmetric, larger on the side of this innervation, and hyperplastic in its motor region (11·7–33·7 p.c.).

The spinal enlargement and hyperplasia on the side and level of the supplementary innervation existed also in certain cases where the grafted limb was not innervated, but where the autochthonous right hindlimb was doubled in its distal part, beginning with the tibia, the two distal enantiomorphs being innervated.

When the total volume of the innervated limbs, autochthonous and grafted, on the operated side, was sensibly the same as that of the normal contralateral hindlimb, the cord was symmetrical, but hyperplastic (8 p.c.) locally, in its motor region, on the side of the graft, at the level of the latter's innervation.

The motor hyperplasia of the cord due to the emission of supplementary nerves was accompanied by an augmentation in weight (7·8–21·8 p.c.) of the same motor region.

A second series of operations consisted in ablations of the right hindlimb rudiment, at the tail-bud stage, and its replacement by the skin of a second embryo, so as to hinder the formation of the limb. In these cases the right hindlimb did not form, or else was very small and deformed. In all cases the lumbo-sacral plexus did not exist on the side of the ablation.

The spinal cord was then reduced and hypoplastic in its motor region (10·7–20·3 p.c.) on the side and level from which the lumbo-sacral plexus was absent, and there was no motor horn on that side and level. In some cases where the small and deformed limb was innervated, this feeble

innervation did not prevent the reduction of the cord, its motor hypoplasia (8·6 p.c.), nor the absence of the motor horn on that side and level.

Thus, any peripheral surcharge, due either to the innervation of a supernumerary hindlimb or to the innervation of a double limb, gives rise to a motor hyperplasia and an enlargement of the cord on the affected side and level. On the other hand, any decrease in the innervation, due to the total absence of a plexus or to its strong diminution, reverberates on the affected side and level of the cord by a motor hypoplasia, the absence of the motor horn, and a reduction in size [May, 1933].

These results concerning the motor neurones, added to those observed by other authors and by ourselves [1932] in the case of the sensory neurones, lead to the statement of the following laws:

1. There is no normal development of the spinal cord, sensory or motor, without the action exerted by the stimulations of the peripheral organs.

2. In development, the bilateral equality of the sensory and motor neurones depends closely on the bilateral equality of innervation of the peripheral organs.

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PROCEEDINGS
OF THE
PHYSIOLOGICAL SOCIETY,
May 12, 1934.

Two instruments for recording the movements of the intestinal sphincters and of the stomach. By G. NORMAN MYERS, *Beit Memorial Research Fellow.* (*Pharmacological Laboratory, Cambridge.*)

Elliott [1904] describes a simple piece of apparatus for recording the movements of the alimentary sphincters. This has been modified so as to make it more rigid and easier to manipulate into position within the sphincter. A diagram of this apparatus is shown in Fig. 1 which is self-

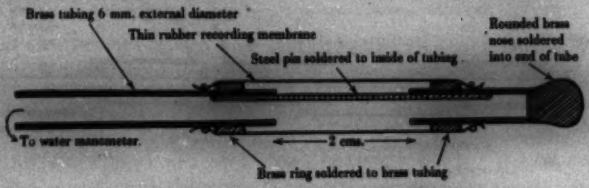
Brass tubing 6 mm. external diameter
Thin rubber recording membrane
Steel pin soldered to inside of tubing
Rounded brass nose soldered into end of tube
To water manometer.
2 cms.
Brass ring soldered to brass tubing

Fig. 1. Apparatus for recording pyloric movements.

explanatory. Elliott suggested the use of the dried external jugular vein of a dog as a covering membrane. Constriction and relaxation will then be registered only when occurring in the muscular wall over the 20 mm. in contact with the membrane. This type of membrane gives excellent results but is not always to hand. A thin rubber membrane such as the end of a small finger stall gives almost equally good results if not drawn too tightly across the gap.

The apparatus is filled with water and connected to a water manometer to indicate the pressure under which the membrane is working. The movements of the water column are then recorded by connecting the air above its surface to a closed system with a delicate tambour. The pressure changes are so slight that the apparatus practically gives readings of volume changes under constant pressure.

The animals are anæsthetized with ether, pithed and the pyloric or ileo-colic sphincter isolated. The apparatus is passed through an artificial

opening in the gut wall until it lies within the embrace of the sphincter muscle. The open end of the gut is ligatured to prevent the escape of the intestinal contents. The intestine is covered with omentum and the abdominal walls drawn loosely together.

Fig. 2 shows a further modification by which the movements of the stomach and pyloric sphincter can be recorded simultaneously, using only one instrument. It is virtually the sphincter movement recorder shown in Fig. 1 with the addition of a by-pass for the balloon recorder which is placed in the stomach. The instrument is filled with water and both the side arm (pyloric recorder) and the straight arm (stomach recorder) connected to water manometers which are connected, in turn, to delicate recording tambours. The apparatus is passed through an artificial opening in the duodenum until the balloon rests within the stomach cavity and adjusted until the membrane lies within the sphincter. The apparatus is

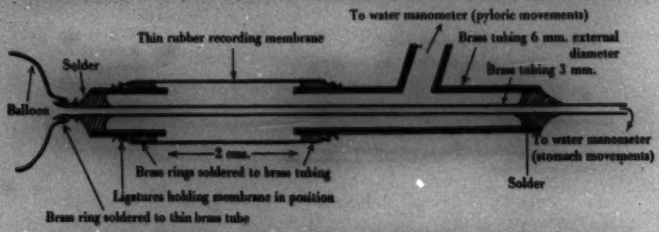

Fig. 2. Apparatus for recording stomach and pyloric movements simultaneously.

ligatured into the proximal part of the duodenum while the distal end is closed with a second ligature.

These instruments are easy to handle and have given satisfactory results over a long period of use.

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Lactic acid formation in the muscles of adrenalectomized animals. By D. NACHMANSOHN. (*Sorbonne, Paris.*)

The formation of lactic acid in the gastrocnemius of the guinea-pig is considerably diminished in adrenalectomized animals. The isometric coefficient of lactic acid for the normal muscle is 116×10^{-6} as the average

of thirty experiments (variation from 89 to 165×10^{-6}). This is of the same order as for the frog's gastrocnemius. Variation of temperature between 37° and 25° does not influence the coefficient, although the number of twitches and the tension developed are less at the lower temperature. The coefficient for the muscle of adrenalectomized animals is considerably higher, but only when the animal reaches a state of adrenal insufficiency, not immediately after the operation, which does not influence the coefficient. Shortly before the death of the animal the coefficient increases very greatly (to 400, 500, 600 and more) although the tension developed is not much diminished. This effect is not due to the disappearance of glycogen. It can be reversed by injections of adrenaline, and of cortico-adrenal extract made according to the methods of Swingle and Pfiffner.

These experiments were made in collaboration with G. Kuschinsky.

Distribution of choline and acetylcholine in suprarenal glands. By W. Feldberg and H. Schild. (*National Institute for Medical Research, Hampstead, London, N.W.* 3.)

The presence of choline in the suprarenal glands was recorded by Reid Hunt [1899, 1901], and by Lohmann [1907]; the latter showed that most of it came from the cortex. Though the suspicion that his suprarenal extracts contained an unstable and more active derivative of choline led Reid Hunt to discover the remarkable activity of artificial acetylcholine, the natural presence of this substance in the suprarenal gland has only been detected in recent years [Minz, 1932; Chang and Gaddum, 1933; Plattner, 1934] by the more sensitive and specific physiological tests now available. The question whether this physiologically recognized acetylcholine is contained in the cortex or the medulla of the gland has not hitherto been investigated; and it has recently gained interest from the evidence that acetylcholine is liberated as the transmitter of nerve impulses to the medullary cells [Feldberg and Minz, 1933; Feldberg, Minz and Tsudzimura, 1933].

We have made extracts from the separated cortical and medullary tissue of a number of horse and ox suprarenals and estimated their acetylcholine contents, testing them on preparations of the leech body wall and of the frog's rectus abdominis muscle, both sensitized with eserine, in comparison with standard dilutions of acetylcholine. The choline contents were also estimated, in some cases, by evaporating the

extracts to dryness, acetylating the residues, and then testing for acetylcholine. The choline contents being thus known, corresponding amounts of choline were added to the standard acetylcholine dilutions used for comparative estimations of the natural acetylcholine. The glands were obtained as early as possible after slaughtering (1–3 hours). Small pieces of pure cortical and pure medullary tissue were dissected from the same gland. The extracts were made by finely mincing the samples in cold 10 p.c. trichloracetic acid. Excess of the acid was removed by shaking with successive portions of ether, and the extract was then evaporated to dryness. From the residue the choline and acetylcholine were extracted with absolute alcohol. This was removed by renewed evaporation, and the final residue taken up with Ringer's solution for testing.

The results are given in tabular form below. The figures in brackets were obtained with the frog's rectus, the others with the leech muscle preparation.

TABLE I.

	Acetylcholine equivalent in γ per g. tissue		Choline content in γ per g. tissue	
Animal	Medulla	Cortex	Medulla	Cortex
Horse	0·53 (0·58)	0·2 (0·26)	—	—
Horse	— (0·38)	— (0·14)	—	—
Horse	0·45 —	0·1 (0·1)	50	350
Ox	0·72 (0·6)	0·2 (0·12)	50	170

The results show that acetylcholine is regularly more abundant in the medulla than in the cortex, the proportion varying from 2·5:1 to 4·5:1. The reverse relation holds with regard to the choline. The choline is present in much greater quantity, though the acetylcholine in the medullary extracts still accounts for most of their activity. The fact that our figures for acetylcholine content are lower than those of some other observers may be due to the re-extraction of the original extract with alcohol. This probably entails some loss, but it is necessary to eliminate the complicating action of excess of potassium ions in the biological tests.

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Action of eserine on transmission through the superior cervical ganglion. By W. Feldberg and A. Vartiainen. (*National Institute for Medical Research, Hampstead, London, N.W.* 3.)

In a recent communication Feldberg and Gaddum [1933] recorded observations suggesting that acetylcholine acts as chemical transmitter of impulses at the synapses in the superior cervical ganglion. The need of eserine to protect the acetylcholine on its way out of the ganglion was emphasized, but the effect of eserine on the transmission of the nervous action was not discussed. A few experiments had shown that perfusion of eserine in sufficient concentrations soon caused a progressively depressant effect on transmission through the ganglion; and this effect had complicated and made of doubtful significance the appearance of an initial potentiation. Eccles [1934], in his recent experiments with electrical recording of ganglionic and postganglionic impulses, detects only this depressant effect of eserine, and concludes that liberation of acetylcholine "is therefore not responsible for the transmission of impulses through the ganglion."

We have now observed that, when a high dilution (1 in 10^6) of eserine is briefly perfused through the ganglion, the contractions of the nictitating membrane, produced by a regular series of equal groups of submaximal preganglionic stimuli, show a pronounced potentiation by the eserine. When a stronger solution of eserine (1 in 2×10^4–10^5) is perfused through the ganglion, the effect of preganglionic stimulation is rapidly depressed; but so also is the stimulating effect on the ganglion cells of small doses of acetylcholine injected into the perfusion. Responses of the ganglion to these two kinds of stimulation recover together as the excess of eserine is washed out. The effects of eserine, when thus applied, are accordingly in favour of the view that liberation of acetylcholine is responsible for the transmission of impulses through the ganglion.

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Chemical transmission at motor nerve endings in voluntary muscle? By H. H. Dale and W. Feldberg. (*National Institute for Medical Research, Hampstead, London, N.W.* 3.)

Acetylcholine appears to act as transmitter of impulses at ganglionic synapses in virtue of its "nicotine" action. The same aspect of its action makes it a potent stimulator of certain normal voluntary muscles in lower

vertebrates, and of all those of mammals when their motor nerves are degenerated. This action raises the question, whether transmission at a motor nerve ending may be of the same type as that at a ganglionic synapse. Several observers [Hess, 1923; Shimidzu, 1926; Brinkman and Ruiter, 1924, 1925] have recorded the liberation from a perfused voluntary muscle (frog) of something like acetylcholine, when the nerve was stimulated. The nerve, however, has in all cases been a mixed one, containing sensory and sympathetic fibres, as well as motor fibres to the striated muscle; neither the identification of the substance nor the meaning of its appearance has been clear.

We have perfused the tongue of the cat with warm, oxygenated Locke's solution containing a little eserine. On rhythmically stimulating the (motor) hypoglossal nerve, we have regularly observed the appearance in the venous fluid, inactive in the resting intervals, of a substance having the usual properties by which we now recognize acetylcholine. The effect is unaltered by degeneration of the sympathetic fibres which join the hypoglossal nerve. Similar results have been obtained with leg muscles (dog) perfused through the popliteal vessels, with excitation of ventral spinal roots, after removal of the lumbar sympathetic chain.

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The chemical transmitter of nervous stimuli to the sweat glands of the cat. By H. H. Dale and W. Feldberg. (*National Institute for Medical Research, Hampstead, London, N.W.* 3.)

The hind foot of a cat is perfused with warm, oxygenated Locke's solution containing a little eserine (1 in 7×10^5), and the venous effluent is tested for activity. In the resting condition no significant activity is detected. When the lower end of the abdominal sympathetic chain is stimulated, causing secretion of sweat on the hairless pads, a substance appears in the venous fluid indistinguishable from acetylcholine in its actions on the eserinized leech muscle, frog's heart and cat's arterial pressure, and in its rapid inactivation in alkaline solution. If the hairless pads, with the sweat glands, are excluded from the perfusion, no acetylcholine appears on sympathetic stimulation. The postganglionic nerve

fibres to the sweat glands, though anatomically sympathetic, are therefore cholinergic, as had been predicted [Dale, 1933]. Pharmacological evidence appears to indicate that the same is true of the nerve supply to the sweat glands of man, though not of some other animals.

Note. Finding by accident that contact with the palm and fingers of the human hand imparts to Ringer's solution a powerful stimulant action on leech muscle, we suspected acetylcholine in human sweat. A few tests showed that the substance concerned is not acetylcholine, and its nature will be further investigated. We mention the point now as a warning to those using leech muscle as a test object. If small volumes of solution are mixed by shaking in a tube closed by a finger, they readily acquire an activity which may be misleading.

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Threshold and current distribution in nerve.

By W. A. H. Rushton. (*University College, London.*)

A uniform, unbranching stretch of frog's nerve 25–30 mm. long was set up in air in a moist chamber. Threshold and sub-threshold constant currents 0·8σ duration were applied through non-polarizable calomel electrodes each meeting the nerve approximately at a point, the cathode being fixed and the anode movable. Measurements of threshold current and voltage were made for various distances between the electrodes, and hence the resistance found for various lengths of nerve. The current was found by the ballistic throw of a Downing moving-magnet galvanometer of high sensitivity (kindly lent by Prof. A. V. Hill).

The curve of the resistance of nerve plotted (vertically) against the length of nerve (horizontally) between the electrodes is characteristic. For lengths above 10 mm. the relation is linear, but for shorter lengths the resistance drops more and more below this line; *i.e.* the slope of the line which for great lengths is constant, increases continually with decreasing interpolar distance. This result is to be expected from the "kernleiter" structure of nerve, since the core can hardly contribute to the conduction at very short lengths, hence the resistance per length is greater in these circumstances. From this resistance-length curve it is possible to find the values of the three resistances which determine the current distribution in nerve, namely, the core, the insulating sheath and the surrounding interstitial fluid. Knowing these three resistances, the

distance apart of the electrodes, and the current in the galvanometer, we may calculate the current in any part of the nerve, and in particular the current density across the insulating sheath at the cathode, which is that fraction of the current which probably stimulates.

The main object of this experiment in fact was to find by purely physical measurements what variation in this "stimulating fraction" occurred with different interpolar lengths, and the significant result was obtained that this fraction remained constant. We may conclude, therefore, that the long-known variation of threshold with interpolar length is simply dependent upon the current distribution in the nerve. The shorter the length, the smaller the fraction which penetrates, and hence the greater the applied current required to maintain the stimulating fraction constant.

Cholinergic nature of sympathetic vaso-dilator fibres.

By Edith Bülbring and J. H. Burn.

One of us has previously shown [Burn, 1932] that when the hinder portion of the body of the eviscerated dog is perfused with defibrinated blood containing a certain concentration of adrenaline, then stimulation of the lumbar sympathetic chain is often followed by vaso-dilatation. It has been found that the same effect is observed after removal of the skin, so that the vaso-dilatation must occur in the vessels supplying the muscles. Sir Henry Dale suggested to us the possibility that this action might be cholinergic, and that the fall of blood-pressure might be due to the liberation from the vaso-dilator nerve endings of a substance resembling acetylcholine.

We find that the fall of blood-pressure is increased by eserine. When the effect of sympathetic stimulation is vaso-constriction, then after eserine the same stimulation may give vaso-dilatation. The vaso-dilator action is abolished by atropine. We have, however, not succeeded so far in demonstrating that blood taken from the vein during stimulation contains a substance which stimulates the leech preparation.

We have observed no vaso-dilator effect in cats prepared for perfusion in the same way, but Hinsey and Cutting [1933] have shown that the Sherrington phenomenon [1894] is elicited in the cat by stimulation of postganglionic sympathetic fibres, and we thought it worth while to investigate whether sympathetic stimulation was attended by vaso-dilatation in cats prepared to show the Sherrington phenomenon. We find, as perhaps was to be expected, that in cats taken 9–21 days after

division of the 6th, 7th lumbar and 1st sacral roots, stimulation of the sympathetic chain in the perfused preparation produces only vaso-constriction.

Evidence that the vaso-dilator effects in the dog are not peculiar to the perfused preparation has been obtained in dogs anæsthetized with chloralose after evisceration. If the blood-pressure be raised by slow intravenous infusion of adrenaline or if eserine be injected, stimulation of the lumbar sympathetic chain may result in a fall of the general blood-pressure.

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Liver fat and blood determinations after adrenalectomy. By J. F. B. Barrett and A. T. Wilson. (*Courtauld Institute of Biochemistry and the Department of Physiology, The Middlesex Hospital, London, W.* 1.)

The amount and iodine value of liver fat—and, in addition, certain blood changes—were observed in four sets of three cats. Each set consisted of an animal (lettered A–D below) adrenalectomized in two stages, and two control animals numbered I and II in each set. The first of these (I) was a normal animal; the other (II) had been adrenalectomized on one side, and after an interval a second "dummy" operation had been performed.

All twelve animals were kept initially on the same diet for 3 months. In each set the unilaterally adrenalectomized control (II) was submitted to the "dummy" operation on the day following the second operation on the doubly adrenalectomized animal of that set. Both controls received each day the exact quantity of milk taken the previous day by the experimental animal of their own set.

The doubly adrenalectomized animals were shot on the 5th, 8th, 11th and 16th days. They had by then developed well-marked signs of adrenal insufficiency in each case. The last animal (D) had an accessory gland below the left kidney. Intravenous heparin, to facilitate bleeding, was given 2 hours before the animals were shot. Reducing power before and after fermentation, urea, non-protein nitrogen, and, in two sets of animals, total acetone bodies, were determined in the blood.

Chemical methods employed.

Sugar: Folin Wu. Fermentation—Peters and van Slyke [1932].

Non-protein nitrogen: Folin Wu.

Urea: Beaumont and Dodds [1934].

Iodine values: Dam [1924].

Total acetone bodies: Benedict and Behre [1926].

The liver was minced, weighed, dried at 105° C. over-night, and weighed again. Total solids were expressed as a percentage of fresh liver weight in each case.

The fat content and iodine value of two 5 g. samples were determined for each liver, and expressed as percentage of fresh liver weight. In this process petrol ether (B.P. 40°–50° C.) was employed in a 6-hour Soxhlet extraction after dehydration of the samples over-night with sodium sulphate *in vacuo*. Iodine values of the fatty extracts were determined by a modified Dam's method. All analyses were made in duplicate.

TABLE I.

Animal Days after double adrenalectomy, in brackets	Liver fat g. p.c.	Iodine value	Total solids Liver weight p.c.
A (8 days)	2·3	85	—
Controls A I	2·0	102	—
Controls A II	18·9	90	—
B (5 days)	1·2	109	23
Controls B I	17·2	77	40
Controls B II	3·7	103	33
C (16 days)	2·7	101	25·5
Controls C I	6·1	92	32·3
Controls C II	6·4	93	33·1
D (11 days)	2·2	86	25·6
Controls D I	2·2	103	28·8
Controls D II	3·4	110	29·4

TABLE II.

Animal	Reducing power (as glucose)	Non-fermentable reducing power (as glucose)	Urea	N.P.N.	Total acetone bodies
D (11 days)	57	12	220	140	2·0
Controls D I	90	8	40	36	1·5
Controls D II	76	10	38	38	2·4
C (16 days)	71	16	210	130	3·4
Controls C I	75	10	35	39	2·2
Controls C II	79	13	35	40	2·3

All in mg. per 100 c.c. blood.

The absence of ketosis in groups C and D, both in doubly adrenalectomized and in control animals, is of interest, since it followed after 11 and 16 days on a markedly low diet. In the doubly adrenalectomized animals increased blood viscosity produced an increase in the residual

blood in the liver and a consequent fall in the percentage of total solids as compared with the controls. Even allowing for this factor, these results do not show any correlation in these animals between adrenalectomy and the amount and nature of liver fat.

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The validity of R.Q. determinations in the mammalian heart.

By E. W. H. Cruickshank. (*Department of Physiology, Dalhousie University, Halifax, N.S., Canada.*)

A certain amount of controversy has arisen over the question of the validity of the R.Q. estimation in eviscerated preparations. The idea that an R.Q. of unity, in isolated heart-lung as well as eviscerated preparations, is due to a "blowing-off" of CO_2 by over-ventilation or to a displacement of CO_2 from chemical combination in the blood and tissues by accumulating lactic acid, and that it is therefore an unreliable guide to the nature of oxidative metabolism of cardiac or skeletal muscle has been supported by many [Kilborn, 1928; Bornstein, 1929; Ferguson, Irving and Plewes, 1930; Anderson, Cleghorn, Macleod and Petersen, 1931; Petersen, 1933]. On the other hand, evidence is not wanting that the R.Q. of the eviscerated spinal preparation is always unity provided that blood sugar be maintained and over-ventilation excluded [Bornstein, 1929; Corkill, Dale and Marks, 1930]. For artificial respiration a two-chambered pump apparatus with rubber diaphragms as pistons is not entirely satisfactory, since the two chambers maintain a balanced input of air only if the external resistance, *i.e.* the elasticity of the lungs, and the stroke remain constant. Respiration pumps of this type can be made to simulate the production or utilization of oxygen, and are therefore a source of large experimental errors. In all experiments on the R.Q. we have used the Starling Ideal pump, working at a rate of 12–15 strokes per min. and having a stroke volume of 80–120 c.c. By means of a very finely balanced spirometer, with a factor of 4·90 c.c./mm. accurate records of oxygen consumption are obtained. Temperature regulation is strictly watched; CO_2 and moisture are absorbed by soda lime and acid in William's bottles. The Douglas bag method for determining re-

spiratory metabolism is, in our hands, not so accurate as that which makes use of a CO_2 and moisture-absorbing system. The CO_2 of the blood was estimated by the van Slyke manometric apparatus.

Since the disagreement concerning the R.Q. of the eviscerate preparation may throw doubt on the accuracy of the R.Q. obtained in isolated heart-lung experiments, it was thought advisable to place on record the effect, upon the R.Q. of the heart, of changes in blood CO_2 and to show how by simple precautions errors are avoided.

TABLE I.

Period 30 min.	CO_2 c.c.	O_2 c.c.	R.Q.	Blood CO_2 c.c. p.c.	CO_2 c.c. lost	Blood vol. c.c.	R.Q. (corr.)
A. Constancy of CO_2 production and the R.Q. after one hour allowed for gaseous equilibrium.							
1	193·42	190·00	1·02	20·32	2·13	820	1·00
2	188·33	185·50	1·07	20·06	3·12	800	0·99
3	188·33	185·50	1·00	19·57	2·34	780	1·00
				19·27			
B. Records commenced immediately after isolation of heart.							
1	137·43	106·32	1·29	18·90	29·40	700	1·06
2	132·34	115·18	1·15	14·70	15·64	660	1·01
3	111·98	110·75	1·11	12·33	2·08	580	0·99
				11·97			
C. Records with insulin and adrenaline, delay 30 min.							
1	188·30	169·63	1·10	25·01	16·48	820	1·01
2	132·34	185·22	0·71	23·00	+3·66	780	0·73
3	137·43	192·04	0·71	22·47	+2·02	750	0·72
				23·74			

In the first two examples given the blood sugar was maintained at the normal figure, in the third the blood was practically sugar free at the end of the second period. The time stated for gaseous equilibrium is taken from the commencement of the isolation of the heart. An examination of these results clearly indicates that gaseous equilibrium is attained in 45–60 min.; that under conditions of maintained blood sugar the R.Q. of the heart is unity; and that any change in the R.Q. that may arise from a washing out of CO_2 may be avoided or, if that be impossible, corrected for by estimating the CO_2 content of the blood.

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Inhibition of the off-effect in the optic nerve and its relation to the equivalent phase of the retinal response. By RAGNAR GRANIT and P. O. THERMAN. (*Physiology Institute, Helsingfors.*)

It has been shown by Granit and Riddell [1934] that the first effect of a flash of light, delivered during the off-effect of the retinal action potential of a light-adapted frog's eye, is a large negative dip, the size of which increases and diminishes with the level of the (positive) off-effect above the base line. The negative dip is followed by a positive swing. Granit and Riddell further showed that this negative dip is identical with the likewise negative *a*-wave initiating every response to light and that these negative deflections are parts of the large negative component, P III [Granit, 1933], of the retinal action potential.

The off-effect in the retinal action potential is mirrored in the optic nerve as a renewed outburst of impulses at the cessation of the illumination [Adrian and Matthews, 1927]. This makes it particularly interesting to find out what happens in the nerve when a flash of light temporarily suppresses the off-effect in the retina, as it does by activating the negative component. Are the impulses temporarily inhibited?

Using a Cossor cathode ray oscillograph with a condenser-coupled amplifier we have recorded (monophasically) the impulses in the optic nerve of the large Hungarian frog (*R. esculenta*). Light-adaptation and strong stimuli have been used as these are optimal conditions for the effect described by Granit and Riddell.

Controls: For nerve records a condenser of 0·02 μF has been used in the input of the second stage of the amplifier which then becomes too rapid to give more than a minute deflection at "on" and "off" with direct recording of the slow retinal action potential from electrodes on the excised bulb. There is no leakage of the retinal response to the electrodes on the nerve as can be shown by lightly pinching the nerve in front of the electrodes and as near to the bulb as possible. The reaction to light disappears completely from the nerve whereas the retina still is as active as before (direct retinal recording with large condensers).

The upper record of Fig. 1 shows the off-effect in the nerve. The cessation of the illumination is marked by an electric artefact in the oscillograph record and also by the end of the line drawn in Indian ink to the left in the figure. After a latent period of 35–40σ there follows a very briak discharge in which numerous fibres take part, to judge from the displacement of the cathode ray upwards in the picture. There are small oscillations and somewhat irregularly synchronized impulses which continue to form waves in the record for some time in the dark.

The lower record starts in the same fashion with the off-effect in the nerve, but after an interval of 120σ there follows a flash with a duration of 120σ. The first effect of the flash is actually to inhibit the activity in the nerve. There is a rapid drop towards the base line, the waves of grouped impulses disappear and the small oscillations become less definite and may even be absent. The inhibition is followed by a phase of renewed activity in the nerve.

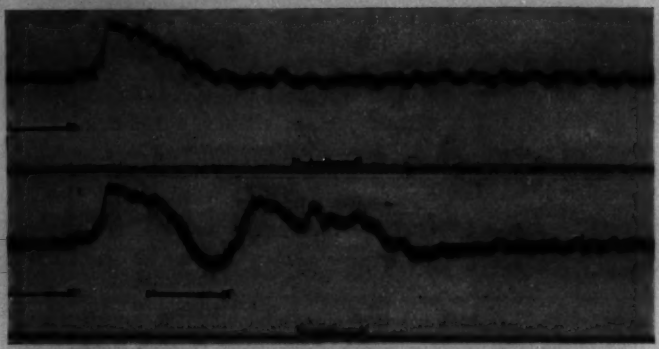

Fig. 1. Records of impulses in the optic nerve of the frog. Lines in Indian ink mark illuminated periods, also marked by electric artefacts in the oscillograph records. Tuning fork of 100 periods is marking the time; an interval of 100σ marked in Indian ink just above time record. Explanation in text.

Thus, with strong stimuli, the first effect of a flash upon the off-effect of the retinal action potential is a large negative dip, the equivalent effect in the nerve is cessation or diminution of the discharge. In the retina the negative dip is followed by a positive swing, the equivalent effect in the nerve is renewal of the discharge.

In view of the fact that the retina ontogenetically is a nervous centre it is interesting to find that slow retinal potentials of opposite sign in some way are associated with the opposite effects of excitation and inhibition.

The results confirm the suggestion [Granit, 1933] that the negative component, P III, of the retinal action potential is associated with inhibition.

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